

KRISTEL EHALA-ALEKSEJEV

The associations between
body composition, obesity and
obesity-related health and
lifestyle conditions with
male reproductive function



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UNIVERSITY OF TARTU
Press

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Estonia

This dissertation has been accepted for the requirement for the degree of Doctor
of Philosophy in Medicine on 20 March 2019 by the Council of the Faculty of
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Commencement: 12 June 2019

ISSN 1024-395X
ISBN 978-9949-03-045-3 (print)
ISBN 978-9949-03-046-0 (pdf)

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University of Tartu Press
www.tyk.ee

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1. LIST OF ORIGINAL PUBLICATIONS

1. Ehala-Aleksejev K, Punab M. (2015). The different surrogate measures of adiposity in relation to semen quality and serum reproductive hormone levels among Estonian fertile men. *Andrology*, 3 (2), 225–234.
2. Ehala-Aleksejev K, Punab M. (2016). Serum hepatic enzyme activity in relation to semen quality and serum reproductive hormone levels among Estonian fertile Men. *Andrology*, 4, 152–159
3. Ehala-Aleksejev K, Punab M. (2017). Relationships between total testicular volume, reproductive parameters and surrogate measures of adiposity in men presenting for couple's infertility. *Andrologia*, e12952
4. Ehala-Aleksejev K, Punab M. (2018). The effect of metabolic syndrome on male reproductive health: a cross-sectional study in a group of fertile men and male partners of infertile couples. *PLoS One*, 13(3):e0194395

Contribution of K.E-A to original publications.

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2. ABBREVIATIONS

ALT	alanine aminotransferase
ANCOVA	analysis of covariance
ATP III	Adult Treatment Panel III
AUDIT	the alcohol use disorders identification test
BF%	body fat percentage
BIA	bioelectrical impedance analysis
BMI	body mass index
CI	confidence interval
EAU	European Association of Urology
FM	fertile men
FSH	follicle-stimulating hormone
GGT	gamma-glutamyltransferase
GnRH	gonadotropin releasing hormone
HPG	hypothalamic–pituitary–gonadal axis
E2	Estradiol
MPIC	male partners of infertile couples
MS	metabolic syndrome
NAFLD	nonalcoholic fatty liver disease
NIHD	National Institute for Health Development
ROC	receiver operating characteristics
SHBG	sex hormone binding globulin
TTV	total testicular volume
WC	waist circumference
WHO	World Health Organization
WHR	waist-to-hip ratio
WHtR	waist-to-height ratio

3. INTRODUCTION

Fertility is the natural human capability of producing offspring. For men, fertility is, first of all, having sperm that can fertilize an egg. It has been suggested that sperm counts are declining over the last decades and that these changes might be responsible for a possible decline in fertility rates in the industrialized world. Carlsen and colleagues, in 1992, were the first to show that semen quality had worsened during the 20th century. The review of 61 papers from 1938 to 1990 documented a significant decrease in mean sperm count and in seminal volume (Carlsen et al., 1992). Furthermore, a recent meta-analysis confirmed the decline in sperm counts between 1973 and 2011 (Levine et al., 2017). The overall decline in fertility is possibly related to nutrition, lifestyle, environmental factors, stress level and a variety of other things. Infertility is a worldwide problem, defined by the WHO as the inability of a couple to achieve conception or bring a pregnancy to term after 12 months or more of regular unprotected sexual intercourse (WHO, 2010). In the diagnosis and management of infertility, the fertility status of both partners must be considered, because this might determine the final outcome (Jungwirth et al., 2015). It has been proposed that infertility affects 15% of couples who do not achieve pregnancy within one year and seek medical treatment for infertility (Thonneau et al., 1991; Sharlip et al., 2002). No absolutely firm data are available for Estonia but considering the above-mentioned prevalence, infertility problems might affect up to 15–20 000 families that cannot have a child naturally. Besides, at least 30 million men worldwide are infertile with the highest rates in Africa and Eastern Europe (Agarwal et al., 2015). Male infertility may be a consequence of congenital or acquired urogenital abnormalities but the common type is idiopathic infertility with no identifiable cause (Jungwirth et al., 2015). The calculated region-specific data shows that in 20–70 % of cases, a male-infertility-associated factor is found (Agarwal et al., 2015).

An increase in male infertility worldwide coincides with male obesity in reproductive-age men which has nearly tripled in the past 30 years (Palmer et al., 2012). In the 21st Century, obesity has become an epidemic and is one of the greatest public health challenges. Worldwide obesity has more than doubled since 1980. In 2016, more than 1.9 billion adults, 18 years and older, were overweight. Of these over 650 million were obese (WHO, 2016).

Data regarding male adiposity shows that in the Estonia, 59.9 % of men are classified as overweight. Of these, 19.8 % are obese, with a body mass index above 30 kg/m² (Tekkel M, 2017). Increased body weight has been associated with number of adverse health consequences including infertility (Guh et al., 2009; Pasquali et al., 2007). It has been proposed that men with excess body weight are at increased risk of infertility according to the dose-response relationship between increasing BMI and subfecundity (Nguyen et al. 2007; Ramlau-Hansen et al., 2007). Obesity can affect fertility by causing also hormonal imbalances. In the prospective Massachusetts Male Aging Study,

moving from a non-obese to an obese state resulted in a decline of testosterone levels comparable to that of advancing 10 years in age (Travison et al., 2007). At the same time, obesity is an important modifiable risk factor for a number of diseases. Measures can therefore be taken to reverse both the unhealthy consequences associated with obesity and its negative impact on male fertility. (Cabler, et al, 2010; Du Plessis et al., 2014). However, many factors contribute to the decline in human fertility and the mechanisms linking obesity and adverse reproductive function are still being explored.

The aim of the current thesis was to specify the relationships between body composition, obesity-related disorders, lifestyle and male reproductive parameters.

This study was performed at the Andrology Centre of Tartu University Hospital.

4. REVIEW OF THE LITERATURE

4.1. Anatomy and physiology of the male reproductive system

4.1.1. The male reproductive organs and spermatogenesis

The male reproductive system consists of several organs acting together to produce functional spermatozoa, and to deliver these spermatozoa to the female reproductive tract.

The primary reproductive organs, testicles are located outside the body cavity in the scrotum and contain seminiferous tubules surrounded by tunica albuginea and hormone-producing Leydig cells as interstices between the tubules. According to available data the optimal testicular volume ranges for normal testicular function vary within wide limits, remaining between 14-35 ml, measured with orchidometer (Takahara et al., 1983; Jørgensen et al., 2002; Sakamoto et al., 2008; Stewart et al., 2009; Nieschlag et al., 2010; Andrology Australia, 2014). The testis is the site of spermatogenesis and also the site of androgen synthesis and secretion. Comparing the two ethnic subgroups of young men from Estonia, ethnic Russians have higher median testis size [26 ml (25th–75th: 23–29)] than Estonians [23 ml (25th–75th: 20–25)] (Erenpreiss et al., 2017). However, with respect to male reproductive health, a critical total testicular volume (TTV) that would ensure adequate reproductive function has not been formally established.

The adult male reproductive anatomy includes also epididymis, ductus deferens, seminal vesicles, bulbourethral (Cowper's) glands, prostate gland, and penis.

Spermatozoa, the haploid germ cells, are produced in the testis and undergo maturational changes as they transit the epididymis. Spermatogenesis starts with stem cells division and ends with the maturation of sperm cells, including mitotic proliferation, meiotic division and transformation of haploid germ cells (spermatids) into sperms. The vas deferens transports the spermatozoa from the epididymis to the ejaculatory duct in the prostate. The spermatozoa and secretions of the seminal vesicles empty together, with secretions from the prostate, into the prostatic urethra. Secretions from the bulbourethral gland contribute to the ejaculate as the mixture exits the body through the penile urethra (Seeley et al., 1999; Nieschlag et al., 2010; Robaire & Chan, 2010).

4.1.2. Endocrine and nervous control of the male reproductive tract

The entire male reproductive system is maintained by pituitary gonadotropins and androgens secreted by the testis. The anterior pituitary produces the gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone

(LH), under the control of the hypothalamic gonadotropin-releasing hormone (GnRH). GnRH secretion is in turn under the control of the kisspeptin-GPR54 (metastin) system. FSH is required for the initiation of spermatogenesis, and LH stimulates androgen production by the testicular Leydig cells. The testis requires high concentrations of testosterone to maintain the process of spermatogenesis and the accessory organs are dependent on androgen for proper secretory function. Testosterone is a precursor of two important hormones: through 5 α -reduction it gives rise to the highly biologically active hormone 5 α -dihydrotestosterone, and through aromatization to estradiol. Estrogens influence testosterone effects by acting either synergistically or antagonistically. Steroids in the circulation exist as free or bound to extracellular proteins such as sex hormone binding globulin (SHBG), androgen binding protein and/or albumin. In addition to hormonal control, the reproductive organs are also subject to sympathetic and parasympathetic nervous control. The ejaculation is under sympathetic and erectile function of the penis is under parasympathetic control. (Nieschlag et al., 2010; Robaire & Chan, 2010).

4.2. Semen quality as a marker of male reproductive function

Semen analysis is a panel of tests performed for evaluation of male reproductive function. The World Health Organization has developed a manual for the evaluation of semen which includes the physical appearance of the ejaculate, assessments of sperm count, motility, vitality, morphology, and functional aspects of the sperm and semen sample (Cooper et al., 2009; WHO, 2010). The proposed lower reference limits (5th percentile) for semen parameters are described in Table 1.

Table 1. Lower reference limits (5th centiles and their 95% Confidence Intervals) for main semen characteristics (WHO, 2010).

Parameter	Lower reference limit (CI)
Semen volume (mL)	1.5 (1.4–1.7)
Total sperm number (10 ⁶ /ejaculate)	39 (33–46)
Sperm concentration (10 ⁶ /mL)	15 (12–16)
Total motility (%)	40 (38–42)
Progressive motility (%)	32 (31–34)
Sperm morphology (normal forms, %)	4 (3.0–4.0)

Semen analysis actually shows the functions of many male reproductive organs and glands as it consists of spermatozoa mixed with secretions from the testis, epididymis, prostate gland and seminal vesicles (Robaire & Chan, 2010). Recommended reference values for semen parameters are not the minimum values to define infertility, as men with semen variables outside these reference ranges may be fertile (Sikka & Hellstrom, 2016). Therefore, if the results of semen analysis are normal, according to WHO criteria, then one test is considered sufficient to determine male seminal function. If the results are abnormal in at least two tests, further andrological investigations are indicated (Jungwirth et al., 2015). There are fluctuations in semen parameters from day to day. Because the volume of the seminal fluid may be quite variable, it has been suggested that total sperm number per ejaculate provides a more accurate assessment of testicular function than does sperm concentration, providing information about the testis and the accessory glands (Nieschlag et al., 2010; Robaire & Chan, 2010; WHO, 2010). Comparing the two ethnic subgroups of young men from Estonia, ethnic Estonians have higher median total sperm counts [$235 \times 10^6/\text{ml}$ (25th–75th: 117–380)] than Russians [$208 \times 10^6/\text{ml}$ (25th–75th: 106–340)] (Erenpreiss et al., 2017).

It is important to differentiate between the following: oligozoospermia (spermatozoa <15 million/mL), asthenozoospermia ($<32\%$ progressive motile spermatozoa) and teratozoospermia ($<4\%$ normal forms) (WHO 2010; Jungwirth et al., 2015).

The quality of semen analysis varies depending on how and where it is collected and on the analytical methods employed. There are also factors that usually cannot be modified, such as sperm production by the testicles, secretions of male accessory glands and recent (particularly febrile) illness, as well as other factors, such as abstinence time before the semen analysis, that should be recorded and taken into account in the interpretation of results. Semen quality also depends on the size of the testicles. Several studies have shown a positive correlation between male fertility parameters and testicular volume (Sakamoto et al, 2008a; Bahk et al., 2010; Tijani et al, 2014; Ventimiglia et al., 2016).

4.3. Male factor infertility: diagnostic classification, epidemiology and aetiology

Male infertility may be the symptom of a wide range of disorders and can be classified according to the topographic principle or according to the nature of the cause. However, in many clinics the diagnosis of male infertility still relies solely upon the findings of semen analysis. A scheme for the diagnostic classification of an infertile couple's male partner has been suggested by WHO already a quarter century ago (Rowe et al 1993). This scheme, incorporating the results of semen analysis, clinical examination and hormonal tests, serves as a basis for standardization. According to the recent EAU guidelines, andrological investigations are indicated if semen analysis is abnormal in at least two tests to

define a diagnosis (Jungwirth et al., 2015, 2017). Based on the results obtained, additional tests and procedures may be recommended, including serial semen analyses, endocrine evaluation, post-ejaculatory urinalysis, ultrasonography, specialized tests on semen and sperm, and genetic screening (Pfeifer et al., 2015; Jungwirth et al., 2015, 2017).

A reduction in the male fertility potential may be due to congenital or acquired conditions such as urogenital abnormalities, urogenital tract infections, increased scrotal temperature (e.g. as a consequence of varicocele), genetic abnormalities, endocrine disturbances, testicular failure, immunologic problems, malignancies, systemic diseases, altered lifestyle, and exposure to gonadotoxic factors (Nieschlag et al., 2010; Jungwirth et al., 2015).

The study of Agarwal et al. showed that the percentage of infertile men ranged from 2.5% to 12%. Male infertility rates were highest in Africa and Central/Eastern Europe, whereas corresponding rates for North America, Australia, and Central and Eastern Europe varied from 4.5–6%, 9%, and 8–12%, respectively (Agarwal et al., 2015).

Due to the relative nature of both male and female infertility, it is difficult to define the exact distribution of the causes of fertility, particularly of disturbed male fertility. Males are thought to be solely responsible for 20–30% of infertility cases but contribute to 50% of cases overall. The WHO carried out a multi-center study in 33 centers worldwide involving 7273 infertile couples and found that in 20% of the cases the problem was predominantly in the male, in 39% the problem was predominantly in the female, in 26% abnormalities were found in both partners, and in the remaining 15% no clear-cut cause of infertility was identified (WHO, 1987). A survey carried out among 1686 couples having consulted a gynecologist for an infertility problem detected that in 38% of the cases abnormalities were found in both partners, in 34% of the cases the problem was predominantly in the female and 20% of the cases the problem was predominantly in the male. In that study the origin of couple infertility was not found in 8% of the cases (Thonneau et al., 1991). In addition, Agarwal et al. revealed that the distribution of infertility due to male factors ranges from 20% to 70% (Agarwal et al., 2015).

The cause of fertility impairment cannot be determined in several cases. Part of patients are having a defect in spermatogenesis of which the underlying causes (including genetic ones) remain largely unknown. The only genetic tests routinely used in the diagnosis of male infertility are the analyses for the presence of Yq microdeletions and/or chromosomal abnormalities. Various other single gene or polygenic defects have been proposed to be involved in male fertility. Yet, their causative effect often remains to be proven.

There are several other factors having the potential to influence male fertility such as advanced age, environmental exposures, smoking, drug and alcohol abuse and obesity.

Considering all these factors, we can conclude that the topic is controversial and we still lack a definite answer regarding their role in determining male (in)fertility.

4.4. Male body type and surrogate measures of adiposity

4.4.1. Male body type

It is a well-known fact that there exist two type of fat deposition: male (android or apple type) and female (gynoid or pear type) pattern. Accordingly, men compared to women have a larger visceral fat depot and they tend to deposit excess fat more in the abdominal region (Palmer & Clegg, 2015). The scientific explanations for the difference in body fat distribution between men and women are largely unknown. From a teleologic standpoint, it has been hypothesized that prehistoric man surviving through hunting and quick escape would need an effectively mobilizable energy source thus explaining the predilection for fat storage in the visceral depot due to the fact that visceral fat is more metabolically active and easier to lose (Arner, 1997; Palmer & Clegg, 2015). A healthy body fat range for men is roughly 9-25 %, depending on the age and ethnicity (Gallagher et al., 2000; Hausman et al., 2001; Ho-Pham et al., 2011). A typical western, mostly sedentary lifestyle combined with energy-excess diet and frequently excessive alcohol use favor fat storage. Above the normal range of fat deposition, specially visceral fat, will be a source of proinflammatory cytokines and large amounts of free fatty acids that contribute to insulin resistance and other features of the metabolic syndrome (Fain et al, 2004; Blüher, 2013). Women, with higher levels of subcutaneous fat, are protected from diseases associated with obesity whereas men, with higher amounts of visceral fat deposition, are at increased risk for diseases associated with obesity (Palmer & Clegg, 2015).

4.4.2. Surrogate measures of adiposity

BMI as a surrogate measure of body fat is the standard system for classifying obesity at a population level. There are four categories of BMI ranges: underweight ($\text{BMI} < 18.5 \text{ kg/m}^2$), normal weight ($18.5\text{-}25 \text{ kg/m}^2$), overweight ($25\text{-}29.9 \text{ kg/m}^2$), obese ($\geq 30 \text{ kg/m}^2$) (WHO, 2000). At the same time validity of BMI to distinguish variability in body composition and body fat distribution, have been questioned (MacDonald et al., 2010). A problem with this method is that individuals with a high BMI may be mesomorphic and have a high amount of muscle mass (Cabler, et al, 2010). There are also metabolically obese, normal-weight people who have normal BMI values but who suffer from metabolic complications commonly found in obese (Ruderman & Schneider, 1981). Therefore, BMI may not be the most accurate marker for total body fat percentage (BF%) and an even less suitable tool to assess body fat distribution. There are more accurate surrogate measures of adiposity. First of them is waist circumference (WC). Based on the International Diabetes Federation and WHO definition for Europid men three categories of WC exist: $\text{WC} < 94 \text{ cm}$ (low risk), $\text{WC } 94\text{-}101.9 \text{ cm}$ (increased risk), $\text{WC} \geq 102 \text{ cm}$ (high risk) (Alberti et al., 2009). Second parameter is waist-to-height ratio (WHtR). The systematic

review recommend the use of the boundary value of 0.5 for WHtR supporting the simple public health message “keep your waist circumference to less than half your height” (Browning et al, 2010). A recent study shows that $WHtR \geq 0.5$ indicate early health risk and men are more likely than women to fall into this early ‘increased’ risk category, probably because of their greater propensity to central obesity (Ashwell & Gibson, 2016). Some years ago published meta-analysis has demonstrated that WHtR is statistically superior to WC as well as being superior to BMI in the discrimination of cardiometabolic risk (Ashwell et al., 2012). In addition, bioelectrical impedance analysis (BIA) is a frequently used method for estimating body composition. Unfortunately, no accepted published BF% ranges exist and evidence-based cut-off points for BF% are needed, as exist for BMI (Gallagher et al., 2000; Ho-Pham et al., 2011).

4.5. Lifestyle-related factors associated with male reproductive health

There are several factors that can lead to infertility in men. Although certain causes of male infertility cannot be changed, some causes of can be corrected. Even if at present, there exists no consensus about the effects of common lifestyle factors on male fertility, some of them seem to be more detrimental, such as smoking, alcohol consumption and obesity.

4.5.1. Effects of cigarette smoking on male fertility

Cigarette smoke contains a large number of substances which are recognized as carcinogens and mutagens. Although the effect of cigarette smoking on sperm function has been reported, the mechanisms by which tobacco smoke affects male fertility parameters are not fully understood. Smoking is associated with lower seminal vesicles volume (Lotti et al., 2015), can affect semen quality (Ramlau-Hansen et al., 2007; Joo et al., 2012; Lee et al., 2014; Lotti et al., 2015; Erenpreiss et al., 2017) and cause DNA damage (Anifandis et al., 2014). A positive, dose–response relationship between smoking, testosterone, LH and the LH/free testosterone ratios have been found (Ramlau-Hansen et al., 2007; Wang et al., 2013). It appears that the effects of cigarette smoking on testosterone levels are reversed upon smoking cessation (Wang et al., 2013).

4.5.2. Obesity related changes in male reproductive system

4.5.2.1. The surrogate measures of adiposity in relation to semen quality

Several reports have shown an inverse correlation between BMI and sperm parameters (Jensen et al., 2004; Hammoud et al., 2008; Hofny et al., 2010, Paasch et al., 2010, Leisegang 2014; Bieniek et al., 2016; Taha et al., 2016) and recent meta-analysis concluded that there is a relationship between BMI and sperm quality (Guo et al., 2017). Moreover, proportions of overweight (or obese) patients are significantly increased in groups of patients with azoo- and oligozoospermia (Punab et al., 2017). Also meta-analysis published in 2012 showed that overweight and obesity are associated with an increased prevalence of azoospermia or oligozoospermia (Sermondade et al., 2012). In addition, some researches have reported that WC is inversely related to semen volume (Fejes et al., 2005, Hammiche et al., 2012; Eisenberg et al., 2014), sperm motility (Fejes et al., 2005, Hammiche et al., 2012) and sperm concentration (Hammiche et al., 2012, Leisegang 2014). Tsao CW et al. (2015) showed that increased adiposity (valued by BMI, WC, WHR, WHtR and BF%) was significantly negatively correlated with sperm concentration and normal sperm morphology (Tsao CW et al. 2015). Concurrently, other reports have failed to document association between BMI and sperm parameters (Aggerholm et al., 2008; Li et al., 2009; Duits et al., 2010, MacDonald, 2013) and meta-analysis published in 2010 concluded that there is no evidence of an association between BMI and semen parameters (MacDonald et al., 2010). Further, Lu JC et al. (2014) indicated that obesity-associated markers (BMI, WC, WHR, WHtR) could not predict male semen quality (Lu JC, 2014). To recap, these findings suggest that the impact of male obesity on sperm quality rest controversial and the effects of male obesity on fertility are likely multifactorial (Palmer et al, 2012; McPherson & Lane, 2015).

4.5.2.2. The surrogate measures of adiposity in relation to reproductive hormone levels

Deviations from normal body weight disturb the endocrine system. According to many researches (Jensen et al., 2004; Fejes et al., 2005; Aggerholm et al., 2008; Wu et al., 2008; Chavarro et al., 2010; Rohrmann et al., 2011 MacDonald et al., 2013; Lu JC, 2014; Beaver et al., 2006) and systematic review with meta-analysis (MacDonald et al., 2010), there is strong evidence that BMI, WC and WHtR are negatively associated with testosterone and SHBG levels. In recent years the results of some studies have put into question the long lasting concept of inhibitory effect of hyperinsulinemia on SHBG production and supported the concept that proinflammatory cytokines downregulate SHBG (Simo R, 2012

and 2015). Furthermore, BMI may be positively associated with plasma concentration of estrogens (Kley et al., 1980; Jensen et al., 2004; Chavarro et al., 2010; Rohrmann et al., 2011). It has been proposed that elevated estrogen concentrations may result from an increased conversion of androgens to estrogens by adipose tissue produced aromatase in cases of weight gain (Palmer et al., 2012). Spermatogenesis is under control of gonadotropins but it remains unclear if obesity has an impact on these parameters. Majority of the studies have not found relation between adiposity and gonadotropin levels (Jensen et al., 2004; Fejes et al., 2005; Aggerholm et al., 2008; Chavarro et al., 2010). It has been suggested that many factors could alter hypothalamic-pituitary-gonadal (HPG) axis functioning such as elevated oestrogen concentration (Schneider et al., 1979; Kley et al., 1980), insulin functioning (Salvi et al., 2006), obesity-related cytokines secretion (Fui et al., 2014) and thereby affect male fertility potential. The main associations of hormonal mechanisms by which adiposity affects fertility are described in Figure 1.

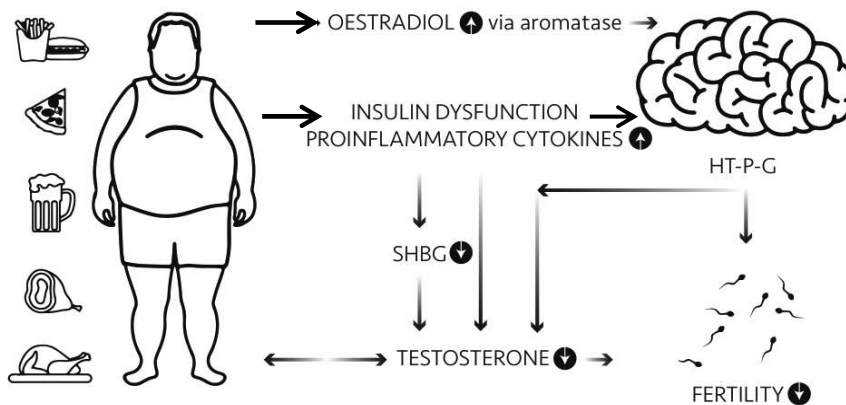


Figure 1. The role of adiposity and potential mechanisms of hormones that may affect male fertility. Weight gain is linked to insulin dysfunction and proinflammatory cytokine production which are potent inhibitors of sex hormone-binding globulin (SHBG) production. Low levels of SHBG are negatively correlated to testosterone levels. The relationship between weight gain and testosterone is complex as low testosterone is both a cause and consequence of adiposity and low testosterone can affect fertility. Adiposity can be also considered a condition of increased aromatization of androgens to oestrogen by adipose tissue. Spermatogenesis is under control of gonadotropins and it has been suggested that elevated oestrogen concentration, insulin functioning and obesity-related cytokines secretion could alter hypothalamic-pituitary-gonadal (HT-P-G) axis functioning and thereby affect negatively male fertility potential.

4.5.3. Alcohol consumption related changes in male reproductive system

When alcohol is added directly to sperm, at concentrations equivalent to those in serum after moderate and heavy drinking, damaging effects are observed in both sperm motility and morphology (Donnelly et al., 1999). Alcohol consumption is associated with increased numbers of morphologically abnormal sperm (Joo et al., 2012) and it has been reported that already modest habitual alcohol consumption of more than 5 units per week had adverse effects on semen quality (Jensen et al.^b, 2014). Also the duration of endocrinological recovery after drinking is a quite long-lasting process and the normal glandular-pituitary feed-back processes may be partly put out of order (Ruusa et al., 1997). It has been shown that compared with non-drinkers current drinkers with increasing recent alcohol intake have lower concentrations of SHBG and possibly higher testosterone and free testosterone levels (Shiels et al., 2010; Jensen^c et al., 2014). At the same time, some other researchers show that alcohol abuse is associated with increased FSH, LH, and estrogen levels and decreased testosterone levels (Muthusami & Chinnaswamy, 2005). Furthermore, men who drink more alcohol also smoke more (Anifandis et al., 2014; Jensen et al.^c, 2014). Besides, in current western societies alcohol and obesity-related health problems are often co-existing and may function in a synergistic manner (Danielsson, 2014).

4.6. Obesity, alcohol consumption and liver injury

The liver, body's second largest organ, plays a central role in metabolic processes. According to epidemiological studies the main cause of liver injury remains alcohol (O'Shea, et al., 2010; Blachier et al., 2013). Even moderate drinking may lead to significant liver enzymes elevations (Danielsson, 2014). Obesity, particularly visceral obesity, can also lead to liver damage. In overweight and obese patients, liver tests, especially alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT), are associated positively with BMI, WC and visceral fat mass. (Burns et al., 1996; Adams et al., 2008; Verrijken et al., 2010; Danielsson et al., 2014). With increasing BMI the effect of moderate alcohol consumption on liver enzymes increases too (Alatalo et al., 2008). Recent studies have shown that serum liver enzymes activity is associated not only with liver injury but also with many cardiovascular disease risk factors and could predict new-onset type 2 diabetes, hypertension, stroke and myocardial infarction (Findeisen et al., 2007; Gomez-Samano et al., 2012; Onat et al., 2012; Labayen et al., 2014).

In addition, liver enzyme levels could be used as clinical predictors of metabolic syndrome (Zhang, et al., 2015).

Still, the data on associations of liver enzymes and incidence of male reproductive health does not exist.

4.7. Metabolic syndrome

4.7.1. Classification of metabolic syndrome

Metabolic syndrome (MS) is a complex of medical conditions characterized by abdominal obesity, dyslipidemia, hypertension and high fasting glucose. In the 1988 Banting Lecture, Reaven used the term syndrome X to refer to the tendency of glucose intolerance, hypertension, low high density lipoprotein (HDL) cholesterol and raised triglycerides, and hyperinsulinaemia to occur in the same individual (Reaven, 1988). Abdominal obesity was not included in Reaven's original description but he suggested that avoiding obesity would protect against insulin resistance. In 1998, the first definition of the MS was formulated by the WHO, which proposed that MS may be defined by the presence of insulin resistance or its surrogates, impaired glucose tolerance or diabetes type 2 combined with at least 2 other factors (hypertension, increased level of blood lipids, obesity and microalbuminuria) (WHO, 1999). Thereafter, various diagnostic criteria of MS have been proposed by different organizations. One of the most widely used criteria of MS is The National Cholesterol Education Program's Adult Treatment Panel III (ATP III) report which identified the MS as a multiplex risk factor for cardiovascular disease (Grundey et al., 2004). MS is diagnosed when a patient has at least 3 of the following 5 conditions: abdominal obesity, elevated triglyceride, reduced HDL cholesterol, elevated blood pressure and elevated fasting glucose. ATP III criteria did not require demonstration of insulin resistance per se. To address variation between professional guidelines, the National Heart, Lung, and Blood Institute (NHLBI), the American Heart Association (AHA), International Diabetes Foundation (IDF) and others have proposed a harmonized definition of MS. It was agreed that there should not be an obligatory component, but that waist measurement would continue to be a useful preliminary screening tool (Alberti et al., 2009). A link between MS and lower general health status of men has also been proved (Lakka HM, et al., 2002; Lotti et al., 2013; Ventimiglia et al., 2016).

4.7.2. Metabolic syndrome related changes in male reproductive system

The worldwide prevalence of MS emerged not only as a predictor of cardiovascular disease but also as a potential contributing factor to male infertility. There is strong evidence that MS is negatively associated with testosterone levels (Corona et al., 2006; Lotti et al., 2013; Ventimiglia et al., 2016a,b) and might be positively related with plasma concentration of estrogen (Maggio et al., 2010). Even if MS and the testosterone deficiency in men are closely linked

then the effect of MS on male semen quality has not been sufficiently investigated on the basis of available data. There are some studies which have concluded that MS may affect male semen parameters (Lotti et al., 2013; Leisegang et al., 2014) et al; Ventimiglia et al., 2016b; Elsamanoudy et al., 2016) and some which deny it (Pilatz et al., 2016; Ventimiglia et al., 2016a).

5. STUDY RATIONALE AND AIMS OF THE RESEARCH

There are several unanswered questions concerning the factors that influence reproductive function in men and the prevalence of obesity and MS has not previously been studied in the context of male infertility in Estonia. Furthermore, the associations between obesity, MS, alcohol consumption and male infertility remain controversial. Most previous studies on male obesity, MS and infertility based mainly on BMI, which is an inaccurate measure of body fat content and does not take into account muscle mass. Data on liver tests and reproductive parameters is so far rather limited.

The general aim of this work was to investigate the eventual role of body composition, obesity and obesity-related health and lifestyle conditions in male infertility assessing their relationship with semen and hormonal parameters in a group of provenly fertile men and male partners of infertile couples.

The specific aims of the research were:

1. To estimate the prevalence and characteristics related to adiposity and MS in a group of provenly fertile men and male partners of infertile couples.
2. To investigate the associations of different surrogate measures of adiposity and MS with basic semen analysis parameters in a group of provenly fertile men men and male partners of infertile couples.
3. To investigate the associations of different surrogate measures of adiposity and MS with serum levels of reproductive hormones in a group of provenly fertile men and male partners of infertile couples.
4. To elucidate which are the best surrogate markers of body composition showing most clearly the relationship between adiposity, semen characteristics and reproductive hormone levels.
5. To examine the relationship between liver tests (GGT, ALT), basic semen parameters and serum levels of reproductive hormones in a group of provenly fertile men.
6. To examine the relationship between alcohol consumption, body composition, serum liver enzyme levels and male reproductive health parameters.

6. SUBJECTS AND METHODS

6.1. Subjects and study design

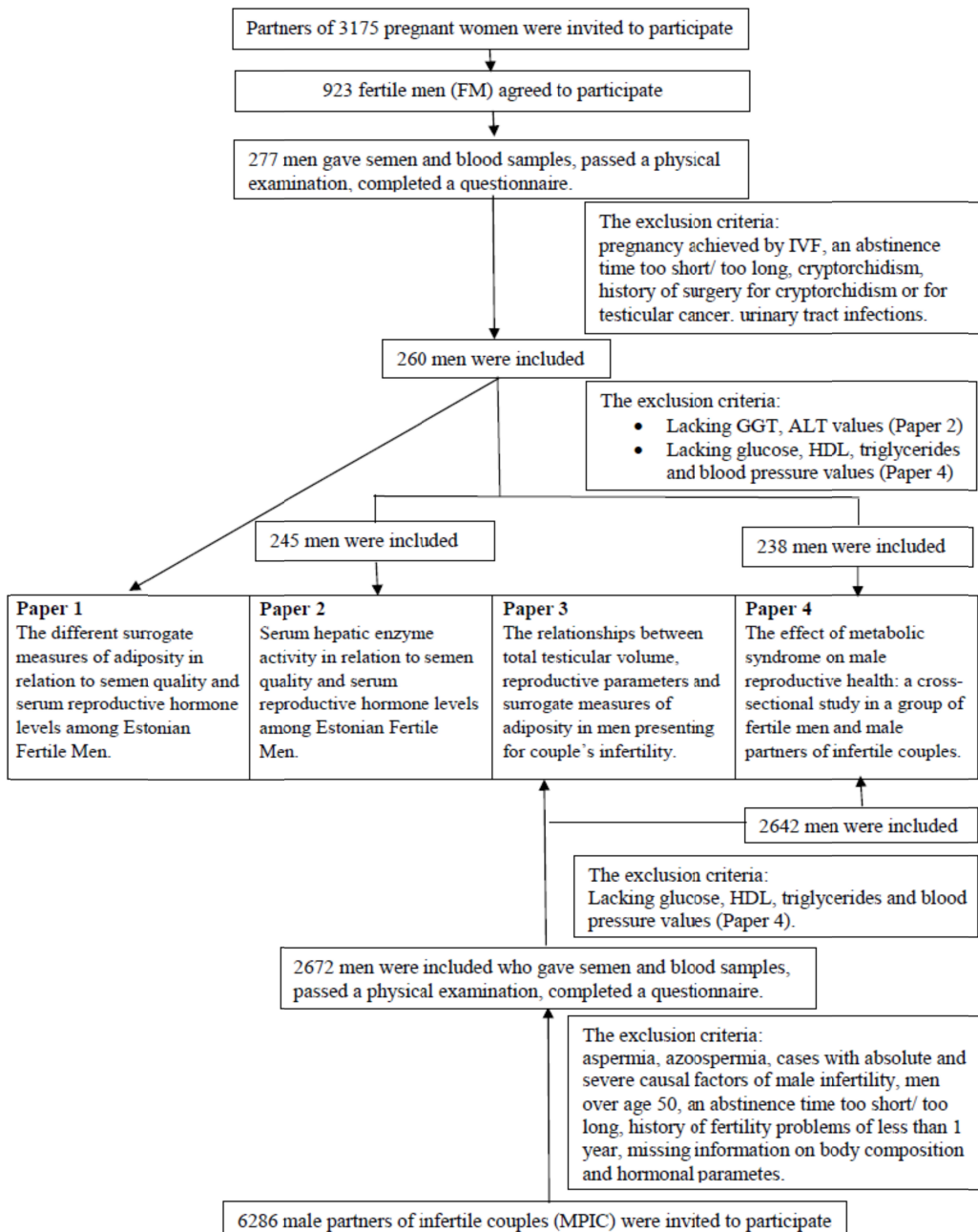


Figure 2. Summary of study subjects.

6.1.1. Male partners of pregnant women

A cross-sectional, multicentre study was conducted during the period 2010 – 2011. 3175 pregnant women who presented for prenatal care at Tartu University Women's Clinic and West-Tallinn Central Hospital Women's Clinic got informed about this study and their partners were invited to participate. Approximately 30% of eligible men agreed to participate. The participants as fertile men (FM) had a choice complete only a questionnaire or in addition to filling out a questionnaire to also pass a physical examination and give blood tests and/or semen analysis. A total 277 men (30 % of participants), who were 21 to 57 years of age, agreed to give semen and blood samples, complete a questionnaire and pass a physical examination. The flow chart (Figure 2) shows the number of subjects recruited and finally included in the studies. Inclusion and exclusion criteria for participants have been described more in detail elsewhere (Paper 1, 2, 4).

In addition, all pregnant partners of liver enzymes and semen quality study's participants were tested for hepatitis B, C and only the men whose partners had negative tests were included (Paper 2).

6.1.1.1. The study of reproductive parameters in relation to adiposity among fertile men

260 men were categorized into 3 BMI groups: (I) $<25 \text{ kg/m}^2$, (II) $25\text{--}29.9 \text{ kg/m}^2$, (III) $\geq 30 \text{ kg/m}^2$ (WHO, 2000). There were no men categorized as underweight ($\text{BMI} < 18.5 \text{ kg/m}^2$) in study group. Men were also divided into 3 groups according to their WC: (I) $<94 \text{ cm}$ (low risk), (II) $94\text{--}101.9 \text{ cm}$ (increased risk), (III) $\geq 102 \text{ cm}$ (high risk). These groups were formed based on the International Diabetes Federation and WHO definition for Europid men (Alberti et al., 2009). According to WHtR, high- and low risk group was formed on the basis of the suggested cut off point: (I) <0.5 ; (II) ≥ 0.5 (Ashwell et al., 2012).

Additionally, we calculated percentiles for BMI [(25th) 23 kg/m^2 , (50th) 25.2 kg/m^2 , (75th) 28.3 kg/m^2], BF% [(25th) 15.7%, (50th) 19.6%, (75th) 23.4%], WC [(25th) 84.0 cm , (50th) 90.0 cm , (75th) 98.0 cm] and WHtR [(25th) 0.463, (50th) 0.497, (75th) 0.540]. Thereafter, we categorized men into quartiles of BMI, BF%, WC and WHtR to explore association between different surrogate measures of adiposity, semen parameters and sex hormones in more detail.

To determine the differences in semen and blood tests parameters according to BMI, BF%, WC and WHtR groups, we took into account known potential confounding factors. In the semen analysis we included study age, sexual abstinence time, smoking and alcohol consumption. In the analysis of blood samples, we controlled for study age, smoking and alcohol consumption.

6.1.1.2. The study of liver tests and alcohol consumption in relation to obesity and reproductive parameters among fertile men

Men were categorized into 3 BMI groups (Table 1, Paper 2) and 3 groups according to their WC as described above. Additionally, we categorized men into quartiles of ALT (n = 244) and GGT (n = 241) to explore association between different liver enzymes, semen parameters and sex hormones in more detail. We calculated percentiles for GGT [(25th) 15 U/L, (50th) 21 U/L, (75th) 35.5 U/L] and ALT [(25th) 17 U/L, (50th) 23 U/L, (75th) 37 U/L].

Men were also divided into three groups according to their weekly alcohol consumption as described in 6.2.2. Besides, 3 groups were formed based on alcohol consumption with or without elevated levels of liver enzymes: (I) non-drinkers, (II) drinkers without elevated levels of liver tests, (III) drinkers with elevated levels of liver tests (ALT and/or GGT). When GGT levels were > 60 U/L and ALT levels were > 41 U/L we considered them as abnormal (by United Laboratories of Tartu University Hospital).

Subsequently we excluded men with only increased ALT levels (n= 7 in the non-drinkers group, n=31 in the drinkers with elevated levels of liver tests group). We compared non-drinkers with elevated ALT with normal levels of ALT, using t-test. We did not find any significant differences comparing data concerning age, reproductive hormones and semen parameters. We repeated analysis, but this time excluding all participants with elevated GGT levels.

Looking at the relationship between reproductive parameters and liver tests we controlled for study age, BMI and alcohol consumption. Looking at the relationship between reproductive parameters, alcohol consumption and liver tests we controlled for study age and BMI.

6.1.2. Male partners of infertile couples.

A cross-sectional, multicentre study was conducted during the period 2008 – 2013 at the Andrology Centres of Tartu University Hospital in Tartu and in Tallinn. Male partners of couples (MPIC) failing to conceive a child over a period of ≥ 12 months, irrespective of their semen analysis results were invited to participate. In addition to the semen analysis, the study subjects went through a structured medical interview and were subjected to physical examinations and blood tests for hormonal analysis. They also had a choice to complete a questionnaire in addition to performed tests. A total of the 6286 males were screened. 2672 men of them (42.5 %), aged 19 to 50, met the eligibility criteria. The flow chart (Figure 2) shows the number of subjects recruited and finally included in the studies. Inclusion and exclusion criteria for participants have been described in detail elsewhere (Punab et al., 2017; Paper 4).

6.1.2.1. The study investigating the relationships between total testicular volume, adiposity measures and reproductive parameters

In order to get a clearer picture of reproductive, anthropometrical and lifestyle findings of men with different total testicular volumes (TTV), participants were divided into 6 groups according to their TTV: (I) < 37 ml, (II) $\geq 37 < 43$ ml, (III) $\geq 43 \leq 46$ ml, (IV) $> 46 \leq 49$ ml, (V) $> 49 < 51$ ml, (VI) ≥ 51 ml. In addition, ROC curve analysis has been performed to determine optimal TTV cutoff value where men would have had normal semen analysis according to WHO references. On the basis of the analysis the obtained cutoff value also corresponds to the mean TTV of the study group: (I) ≤ 46 ml; (II) > 46 ml. Thereafter, we calculated percentiles for BMI [(25th) 23.6 kg/m², (50th) 26.1 kg/m², (75th) 29.1 kg/m²], WC [(25th) 85.5 cm, (50th) 93.0 cm, (75th) 102.0 cm] and WHtR [(25th) 0.472, (50th) 0.514, (75th) 0.561]. We categorized men into quartiles to explore associations between different surrogate measures of adiposity, semen parameters and sex hormones in TTV groups. Potential confounding factors were taken into account. In the semen analysis we included age, sexual abstinence time, alcohol consumption, disorders affecting testicular function, and TTV. In the analysis of blood samples, we controlled for age, disorders affecting testicular function, alcohol consumption and TTV.

6.1.2.2. The study of reproductive parameters in relation to metabolic syndrome in a group of fertile men and male partners of infertile couples

In a total of 238 FM and 2642 MPIC were included in the study (Table 1, Paper 4). To explore the association between MS, semen quality and reproductive hormones, four groups of men were compared: FM-MS⁻, FM-MS⁺, MPIC-MS⁻, MPIC-MS⁺. To investigate the association between MS and reproductive parameters within BMI categories, these study participants were stratified into three standard BMI groups: (I) < 25 kg/m², (II) 25-29.9 kg/m², (III) ≥ 30 kg/m². The analysis was repeated.

MS was defined according to the 2004 updated National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (ATP III) criteria (at least three of the following criteria): waist circumference ≥ 102 cm; triglycerides ≥ 1.7 mmol/L (150 mg/dL) or drug treatment for elevated triglycerides; HDL less than 1.03 mmol/L (40 mg/dL); blood pressure $\geq 130/85$ mm/Hg or use of medication for hypertension; fasting glucose ≥ 5.6 mmol/L (100 mg/dL) or use of medication for hyperglycemia (Grundy SM et al., 2005). When determining the differences in semen and reproductive hormones according to MS, we adjusted for subject's age, smoking, alcohol consumption and TTV.

6.2. Methods

6.2.1. Physical examination

Testicular volumes were measured using the orchidometer (made of birch wood, Pharmacia & Upjohn, Denmark) and expressed in milliliters. The total testes volume was the sum of right and left testicles. The position of the testicles in the scrotum and disorders affecting testicular function [i.e. varicocele (gr 2, 3 and bilateral), testicular trauma, inguinal hernia] were recorded. Examination of patients has been described in detail elsewhere (Punab et al., 2017). A face-to-face clinical interview was conducted to assess the presence of other medical conditions and lifestyle related risk factors. All research personnel underwent the NHANES “Anthropometry Procedures Manual” based training programme (NHANES, 2007). Height was measured by roll-up metal length measuring tape for wall mounting rounded to the nearest 0.1cm and expressed in cm respectively. Body composition was determined using Tanita Body Composition Analyzer (TBF-300MA). Beside weight in kg, actual fat mass in kg and BF% were defined by body analyser. Recommended by Tanita, a standardized BIA protocol was used in order to obtain the most accurate results (Heyward V et al., 1996). BMI was defined as the weight in kilograms divided by the square of the height in meters (kg/m^2). The WC was measured halfway between the iliac crest and the bottom of the 12th costal bone, at the end of normal expiration and expressed in cm. The nurse took two measurements rounded to the nearest 0.1cm and a third if the difference was more than 1 cm. WHtR was defined as waist circumference divided by height, both measured in the same units. The measurement of blood pressure was performed by using the Omron M3W, an electronic digital device for measuring blood pressure in the arm while the patient was seated in a chair for at least five minutes.

6.2.2. Questionnaire

In addition, all men (FM and MPIC) were asked to complete a questionnaire to provide information on medical and reproductive history and lifestyle factors including alcohol and smoking history.

To screen for drinking habits the AUDIT self-reported questionnaire was used (Babor TF et.al, 2001). Men were asked to convert their intake to alcohol units. One unit of alcohol was defined as 10 grams of pure alcohol. Alcohol intake was calculated as the sum of daily reported unit intakes within that week. In addition, the men were asked whether their alcohol intake in the week represented habitual alcohol consumption. They were also asked how often they drink, how many units they consume on one occasion and how many times they consume more than 6 units of alcohol. As Estonia does not have nationally determined explicit limits for weekly alcohol consumption, the daily limits and alcohol-free days were used to derive the cut-off points for this analysis (www.alkoinfo.ee). Men were divided into 3 groups according to their drinking

habits: (I) non-drinkers, (II) moderate drinkers (< 16 units/week), (III) habitual drinkers (\geq 16 units/week).

Men were also divided into 3 groups according to smoking status: (I) non-smokers, (II) former-smokers, (III) smokers.

Medical history included questions about obesity and infertility related chronic diseases such as various endocrinopathies, asthma, neurological disorders, depression, as well as cardiovascular, renal, gastrointestinal and joint diseases.

6.2.3. Semen analysis

Semen quality parameters like semen volume, concentration, total sperm count, motility and morphology were assessed. The analysis of semen samples was performed in accordance to WHO recommendations at the time of recruitment (WHO, 1999; WHO, 2010). Semen samples were obtained by masturbation after a required abstinence period of minimum 2 days and ejaculated into a sterile collection tube in a private room near the laboratory. Artificial lubrication was not allowed for semen collection. After ejaculation, the semen was incubated at 37°C for 30–40 min for liquefaction. The actual period of ejaculation abstinence was calculated in days between current and previous ejaculation as reported by the men. Semen volume was estimated by weighing the collection tube with the semen sample and subsequently subtracting the predetermined weight of the empty tube assuming 1 g = 1 ml. For assessment of the sperm concentration, the samples were diluted in a solution of 0.6 mol/l NaHCO₃ and 0.4% (v/v) formaldehyde in distilled water. The sperm concentration was assessed using the improved Neubauer haemocytometers. Total sperm count was calculated by multiplying semen volume by sperm concentration. The motility assessment was performed in duplicate and the average value was calculated. Motility was assessed in order to report the number of progressively motile spermatozoa (WHO motility classes A + B). Smears for morphology assessment were made. Following fixation and Papanicolaou staining morphology was assessed according to strict criteria (Menkveld et al., 1990). Semen smears were used for detecting white blood cells. The smears were air-dried, Bryan-Leishman stained, and examined with the use of oil immersion microscopy (magnification: $\times 1000$). Polymorphonuclear leukocytes were differentiated from spermatids by the presence of segmented nuclei, bridges between lobes of nucleus, and specific granulation of the cytoplasm. The leukocytospermia was defined according to WHO definition for the neutrophil count > 1million/ml.

The sperm analyses were performed at the Andrology Centres of Tartu University Hospital in Tartu and in Tallinn. Special emphasis was paid to the quality assurance and control procedures with follow WHO guideline (WHO, 2010). The laboratory technicians performing semen analysis in both centres had similar training background and have been participated in several semen analysis standardisation workshops and external quality control schemes

organized in the framework of Nordic-Baltic scientific collaboration. Our technicians performed regular intralaboratory control by examining the same semen samples blindly and the between-technician variation in sperm concentration was constantly <10%.

6.2.4. Blood sampling

Blood samples were taken between 8 am and 10.30 am after an overnight fast same day that the semen sample was produced. Blood was centrifuged and serum was used to determine of testosterone, oestradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), sex hormone binding globulin (SHBG) and insulin concentrations concentrations by chemiluminescence immunoassay (CLIA) method (Immulite 2000, Siemens Healthcare Diagnostics, Deerfield, IL, USA).

Hexokinase method was used for serum glucose, enzymatic colorimetric method was used for HDL cholesterol, triglycerides and GGT assays (Cobas c 501, Roche, Basel, Switzerland) and for ALT a kinetic photometric method were used (Cobas 6000; Roche Diagnostics CH – 6343 Rotkreuz, Switzerland).

6.2.5. Statistical analysis

The assumption of normality was checked for parametric tests. The histogram and the P-P (probability-probability) plot were used to test for normality. The data regarding semen volume, sperm concentration and total sperm count were positively skewed. Skewed data were normalized by the natural log-transformation before analysis. The back-transformed adjusted mean values ensured the most reliable results and were used for evaluation.

Spearman's correlation coefficient was calculated between BMI, BF%, WC and WHtR.

The independent t-test or the analysis of variance (one-way ANOVA model with Bonferroni's multiple t-tests by pairwise group comparison) was applied to test for significant differences between the formed groups and the various parameters (reproductive, anthropometric, lifestyle parameters), with $P < 0.05$ considered statistically significant.

Pearson chi-square test was used to investigate whether distributions of categorical variables differ from one another.

To reduce the error variance and give a clearer picture of the outcome we also took known potential confounding factors into account using analyses of covariance (ANCOVA) with Bonferroni-corrected post-hoc pairwise comparisons.

Missing information on smoking status and alcohol consumption restricted our data set. We compared two groups of men (with whole data and with missing values) using t-test. We did not find any significant differences comparing

data concerning age, testicular volumes, reproductive hormones and semen parameters.

The results are presented as mean (95% confidence intervals [CIs]), median with the 25th and 75th percentiles and as adjusted back-transformed mean values (95% CIs). Data analyses were performed using the SPSS for Windows version 20.0 (SPSS Inc. Chicago, IL). Statistical significance was defined as $p < 0.05$

6.2.6. Ethical considerations

Participation in the study was voluntary. Studies were approved by the Ethics Committee on Human Research of the University of Tartu and written informed consent was obtained from all participants. (certificate 152/4, 2006 and certificate 188/M-16, 2009).

7. RESULTS AND DISCUSSION

7.1. Prevalence and characteristics related to adiposity and metabolic syndrome among the Estonian fertile men and male partners of infertile couples

7.1.1. Prevalence and characteristics related to adiposity (Papers 1, 2, 3, 4)

Men in the MPIC group had a significantly higher mean BMI compared to FM. Central obesity defined by high risk WC (≥ 102 cm) and WHtR (> 0.5) was also more prevalent among these men. Clinical findings are shown in Table 2. In 2016, 59.9% of men in the general population were overweight or obese (Tekkel M, 2017). Compared to the general population less overweight and obese men was found in the FM group (51.2%). The prevalence of overweight and obesity was almost comparable between men in the general population and MPIC (60.4%).

According to BMI categories men with overweight and obesity, in both groups (FM and MPIC), were older ($p=0.009$; $p<0.001$, respectively) and the percentage of overweight and obesity increased with age. 28 (29.8%) of FM and 343 (35.0%) of MPIC under the age of 30 were overweight (BMI $\geq 25<30$) while 9 (9.6%) of FM and 152 (15.5%) of MPIC were obese (BMI ≥ 30). At the same time, over the age of 40, 10 (32.3%) of FM and 144 (46.9%) of MPIC were overweight while 9 (29.0%) of FM and 83 (27.0%) of MPIC were obese. Likewise, the age-related rise in overweight and obesity among men in the general population can be noticed. In 2016, 10.7 % of 25- to 34-year-olds and 27.2 % of 45 to 54-year-olds were obese (Tekkel M, 2017).

From men who self-reported their 5 years earlier weight, 107 (55.2%) of FM and 631 (58.3%) of MPIC gained and 48 (24.7%) of FM and 259 (23.9%) of MPIC maintained weight during the period.

The higher prevalence of adiposity among MPIC has been accompanied by increased levels of liver enzymes (GGT, ALT) compared to FM. However, highly significant positive relationship was also obtained between liver tests and every BMI as well as WC group in FM (Paper 2, $p<0.001$ for both parameters). Likewise, earlier studies have found that BMI, WC and visceral fat mass are positively associated with ALT and GGT levels (Adams et al., 2008; Verrijken et al., 2010; Niemelä et al., 2017).

Table 2. General characteristics of study groups.

	FM	MPIC	P
	(n =260)	(n =2672)	
Personal characteristics, mean ± SD			
Age in year	32.3 (± 6.7)	32.6 (±5.7)	.475
Height (cm)	180.5 (± 6.1)	181.3 (±6.9)	.065
BMI (kg/m ²)	25.7 (±3.8)	26.6 (±4.5)	.009 χ^2
<25 n (%)	127 (48.8%)	1056 (39.6%)	
≥25<30 n (%)	95 (36.5%)	1083 (40.5%)	
≥30 n (%)	38 (14.7%)	533 (19.9%)	
BMI 5 years ago ^a (kg/m ²)	24.7 (±3.6)	25.1 (±3.9)	.142
Waist circumference (cm)	91.0 (±9.7)	94.3 (±12.4)	<.001 χ^2
<94 n (%)	165 (63.5%)	1343 (50.3%)	
≥94<102 n (%)	57 (21.9 %)	631 (23.6 %)	
≥102 n (%)	38 (14.6 %)	698 (26.1%)	
Waist-to-height ratio	0.504 (± 0.055)	0.521(±0.067)	.002 χ^2
≤0.5 n (%)	136 (52.3%)	1128 (42.2%)	
>0.5 n (%)	124 (47.7%)	1544 (57.8%)	
Health and lifestyle characteristics, n (%)			
Metabolic syndrome ^b	29 (12.2%)	471 (17.8%)	.028 χ^2
ALAT U/L	30.1 (21.1%)	33.4 (24.2%)	.043
GGT U/L	28.9 (21.0%)	34.6 (40.6%)	.035
Smoking status ^c			<.001 χ^2
Never smoker	105 (41.8%)	435 (22.1%)	
Former smoker	74 (29.5%)	622 (31.6%)	
Current smoker	72 (28.7%)	914 (46.4%)	
Alcohol status ^d			.036 χ^2
Non alkohol users	45 (18.0%)	230 (12.2%)	
< 16 units/week	162 (64.8%)	1318 (70.0%)	
≥ 16 units/week	43 (17.2%)	334 (17.8%)	
Units per week, mean± SD	7.7 (± 8.2)	8.7(± 11.1)	.176

a FM: 66 missing, MPIC: 1589 missing; **b** FM: 22 missing, MPIC: 30 missing; **c** FM: 10 missing, MPIC: 701 missing; **d** FM 10 missing, MPIC: 790 missing
The independent t-test and Pearson chi-square test were used.

7.1.2. Prevalence and characteristics related to metabolic syndrome (Paper 4)

To the best of our knowledge, this study was the first to report a significantly higher prevalence of MS in MPIC compared to FM of the same age range (Table 2). The observed difference was 5.6 %. Our findings showed that 17.8% of MPIC presenting for couples' infertility met the criteria for MS of which the rate is higher than in previously reported findings (Lotti et al., 2013; Ventimiglia et al., 2016a,b). The difference between studies may come from the used definition and the prevalence of MS differing across countries as shown by Scuteri et al., (2015). We chose the adapted NCEP-ATP III criteria to define MS because this definition does not require insulin resistance or central obesity as a necessary diagnostic component and is widely used. The prevalence of MS among men in the general population was higher compared with our results. MS was found in 25.7 % of 20- to 44-year-olds men and 28.2 % of 45 to 60-year-olds men (Eglit et al., 2012).

When stratifying FM and MPIC according to the presence or non-presence of MS men with MS, in both groups, were centrally obese as indicated by increased WC, WHtR and BMI (Table 3). Still, in the presence of MS 3.4% of the FM and 6.8% of the MPIC were categorized as normal-weight (BMI <25 kg/m²). Besides central obesity, other metabolic syndrome indicators (low HDL-cholesterol, elevated triglycerides and blood pressure levels) in both MS⁺ groups have been found (Table 3). However, glucose concentrations were significantly higher only in the MPIC-MS⁺ group. Higher levels of fasting glucose were more frequently found in MPIC-MS⁺ (51.2%) than in FM-MS⁺ (10.3 %). Furthermore, higher insulin levels were found in MPIC-MS⁺ (data available only for MPIC).

Men with MS were older although a significant correlation was seen only in the MPIC group. Irrespective of the definition, the existence of a similar age-profile has been confirmed earlier (Lotti et al., 2013; Ventimiglia et al., 2016).

Interestingly, the most frequent metabolic components in subjects with MS were different between our study and study made by Eglit et al., 2012. Higher triglycerides (93.1% of FM and 80.1% of MPIC), arterial hypertension (78.6% of FM and 77.7% of MPIC) and abdominal obesity (75.9% of FM and 74.3% of MPIC) were the most common abnormalities in our study. At the same time, a population-based cross-sectional study in different counties of Estonia showed that arterial hypertension (94%), abdominal obesity (91%) and impaired glucose metabolism (71%) were the most frequent metabolic components in men with MS (Eglit et al., 2012). It is probable that the prevalence of metabolic variables is dependent on age. On average, men in our study were younger than men in study made by Eglit et al., 2012.

Disorders potentially affecting testicular function and leucocytospermia were not related to MS (Table 3).

Table 3. General characteristics of study groups.

	FM				MPIC			
	no MS	MS	P		no MS	MS	P	
	(n =209)	(n =29)			(n =2171)	(n =471)		
Anthropometric and MS characteristics, mean \pm SD								
BMI (kg/m ²)	25.0 (\pm 3.2)	31.4 (\pm 3.7)	<.001 χ^2		25.6 (\pm 3.8)	31.1 (\pm 4.6)	<.001 χ^2	
<25 n (%)	118 (56.5%)	1 (3.4%)			1015 (46.8%)	32 (6.8%)		
\geq 25<30 n (%)	75 (35.9%)	8 (27.6%)			902 (41.5%)	167 (35.5%)		
\geq 30 n (%)	16 (7.7%)	20 (69.0%)			254 (11.7%)	272 (57.7%)		
Waist-to-height ratio	0.493 (\pm 0.048)	0.582 (\pm 0.045)	<.001		0.506 (\pm 0.058)	0.589 (\pm 0.066)	<.001	
Waist circumference (cm)	89.1 (\pm 8.2)	105.3 (\pm 7.9)	<.001		91.6 (\pm 10.6)	107.2 (\pm 12.1)	<.001	
Blood pressure (mm/Hg)								
Systolic BP	132.0 (\pm 16.2)	141.6 (\pm 16.7)	.004		132.0(\pm 16.3)	144.0 (\pm 15.9)	<.001	
Diastolic BP	78.4 (\pm 9.5)	90.0 (\pm 13.1)	<.001		79.9(\pm 10.0)	90.3 (\pm 10.7)	<.001	
HDLcholesterol (mmol/L)	1.4 (\pm 0.3)	1.0 (\pm 0.3)	<.001		1.4(\pm 0.3)	1.1 (\pm 0.3)	<.001	
Triglycerides (mmol/L)	1.2 (\pm 0.6)	2.6 (\pm 1.1)	<.001		1.2(\pm 0.7)	3.0 (3.1)	<.001	
Fasting glucose (mmol/L)	5.0 (\pm 0.6)	5.1 (\pm 0.5)	.251		5.1(\pm 0.7)	5.7 (\pm 1.6)	<.001	
Fasting insulin (mU/L)					6.6(\pm 5.2)	11.9 (\pm 7.4)	<.001	
Health characteristics, n (%)								
Testicular disorders ^a	35 (16.7%)	6 (20.7%)	.598 χ^2		416 (19.2%)	82 (17.4%)	.378 χ^2	
Leukocytospermia	11 (5.3%)	2 (6.9%)	.717 χ^2		187 (8.6%)	51 (10.8%)	.128 χ^2	

a Testicular disorders: varicocele (gr 2, 3 and bilateral), testicular trauma, inguinal hernia. The independent t-test and Pearson chi-square test were used.

7.2. General characteristics related to reproductive parameters

Overall, 78.5% of FM and 46.9% of MPIC had totally normal semen analysis according to WHO 2010 guidelines. Significantly lower TTV, semen quality (except for semen volume) and higher level of FSH, a common indicator of spermatogenesis impairment, were seen in MPIC compared to FM (Table 4). At the same time testosterone, oestradiol and LH levels did not differ between the two groups.

Besides, the quartile analysis revealed that distributions of total sperm count and sperm concentration in both groups followed inverted J-shaped curve. Even if we did not find statistically significant associations, J-shaped curves appeared between BMI, WC, BF% quartiles and sperm parameters in FM (Figure 3 A, BMI and total sperm count). In MPIC same trend appeared between BMI, WC quartiles and mentioned sperm parameters (Figure 3 B, BMI and total sperm count). Similar associations were already found before between total sperm count (Jensen et al., 2004; Stewart et al., 2009), sperm concentration (Jensen et al., 2004) and BMI.

Table 4. Reproductive parameters of study groups.

	FM	MPIC	P
	(n =260)	(n =2672)	
Total testicular volume (ml)			
Mean (95% CI)	47.7 (46.5; 48.8)	45.9 (45.5; 46.2)	<.004
Abstinent time (day)			
Mean (95% CI)	3.9 (3.7; 4.1)	3.8 (3.7; 3.9)	.245
Semen volume (mL)			
Mean (95% CI)	3.8 (3.6; 4.0)	3.8 (3.7; 3.9)	.899
Sperm concentration ($\times 10^6/\text{mL}$)			
Mean (95% CI)	73.3 (66.4; 81.0)	38.2 (36.3; 40.1)	<.001
Total sperm count ($\times 10^6$)			
Mean (95% CI)	280.3 (252.7; 311.1)	144.0 (136.5; 151.1)	<.001
Motile spermatozoa (%)			
Mean (95% CI)	51.5 (50.0; 52.9)	41.4(40.7; 42.0)	<.001
Normal morphology (%)			
Mean (95% CI)	10.7 (10.1; 11.4)	7.0 (6.8; 7.2)	<.001
Serum FSH (IU/L)			
Mean (95% CI)	3.9 (3.7; 4.2)	4.4 (4.3; 4.5)	.027
Serum LH (IU/L)			
Mean (95% CI)	3.7 (3.5; 4.0)	3.7 (3.6; 3.8)	.951
Testosterone (nmol/L)			
Mean (95% CI)	16.7(15.9; 17.4)	16.6 (16.4; 16.9)	.959
Oestradiol (pmol/L)			
Mean (95% CI)	124.7 (118.5; 130.9)	127.4 (125.3; 129.6)	.427
Health characteristics, n (%)			
Testicular disorders ^a	43 (16.5)	506 (18.9)	.405 χ^2

a Testicular disorders: varicocele (gr 2, 3 and bilateral), testicular trauma, inguinal hernia. The independent t-test was used.

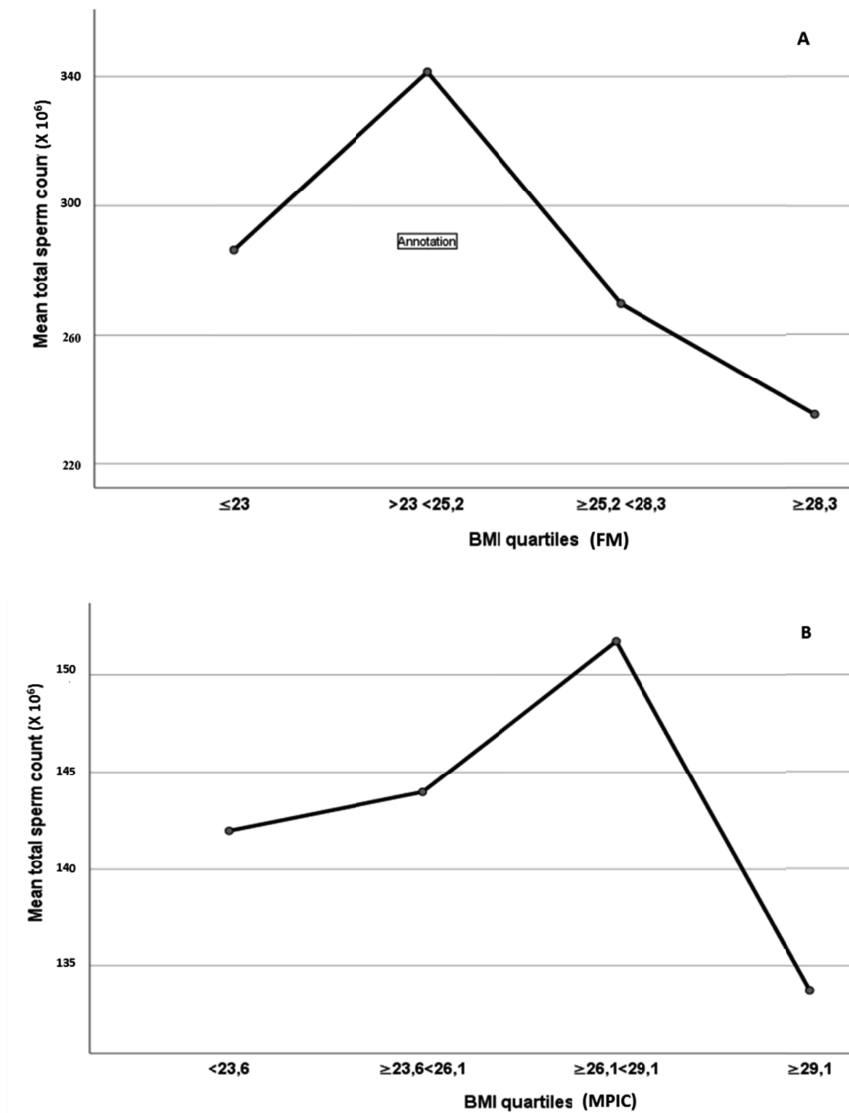


Figure 3. J-shaped curve between BMI and total sperm count in FM (A) and in MPIC (B).

7.3. Obesity and metabolic syndrome related changes in male reproductive health

7.3.1 The different surrogate measures of adiposity in relation to reproductive parameters among Estonian Fertile Men (Paper 1)

After adjustment for covariates a high BF%, WC and WHtR were negatively associated with total sperm count (Table 3, 4, 5 in Paper 1). A post hoc test showed that men in the fourth quarter (BF% ≥ 23.4 ; WC > 98 cm) had a significantly lower total sperm count [217.2 (95% CI: 177.0; 266.7); 208.9 (95% CI: 170.2; 256.7), respectively] compared with men in the second quarter (BF% $\geq 15.7 < 19.6$; WC $\geq 84 \leq 90$ cm) [322.5 (95% CI: 263.0; 395.1) ($p = 0.049$); 329.3 (95% CI: 268.8; 403.4) ($p = 0.014$), respectively]. Similar trend also appeared between the fourth and the third quarter (BF% $\geq 19.6 < 23.4$; WC $> 90 \leq 98$ cm) [317.7 (95% CI: 260.1; 388.0) ($p = 0.05$); 305.2 (95% CI: 249.9; 372.4) ($p = 0.056$), respectively]. The generally accepted cut-off value for the study of adverse effects of WC is 102 cm. It was also confirmed by Hammiche et al. (2012). Based on our analysis; the sensible cut-off value might be smaller, as we show that the effects of WC on total sperm count are seen from a WC > 98 cm. Looking at WHtR, men in the fourth quarter (WHtR ≥ 0.540) had a lower total sperm count [219.0 (95% CI: 178.6; 268.5)] compared this time with men in the first quarter (WHtR < 0.463) [338.0 (95% CI: 274.8; 415.7) ($p = 0.024$)]. Moreover, the BF% was also negatively correlated with semen volume. A post hoc test showed that significant difference appeared between men in the fourth [3.5 (95% CI: 3.1; 3.9)] and the second quarter [4.4 (95% CI: 3.9; 4.8)] ($p = 0.034$).

In addition to the results of our study, other researchers have reported that WC is inversely correlated to semen volume (Fejes et al., 2005, Hammiche et al., 2012; Eisenberg et al., 2014), sperm motility (Fejes et al., 2005, Hammiche et al., 2012) and sperm concentration (Hammiche et al., 2012).

Next to these changes the BMI was not related to sperm parameters. This is in contrast to studies and recent review with meta-analysis that have shown an association between BMI and sperm parameters (Chavarro et al., 2010; Shayeb et al., 2011; Hammiche et al., 2012; Eisenberg et al., 2014; Andersen et al., 2015; Guo et al., 2017).

As demonstrated previously (7.2), distributions of total sperm count and sperm concentration followed inverted J-shaped curve. However, we did not find statistically significant associations between low weight and sperm parameters as did Jensen et al. (2004), but there were not also really underweight men in our study group. At the same time, the curves obtained may explain why we did not find significant differences in sperm count (regarding BF% and WC) between men in the fourth and in the first quarter.

As regards the hormone parameters, in line with other studies, all adiposity markers were negatively related to SHBG and total testosterone levels ($p < 0.001$

for all). Low testosterone levels linked to obesity is confirmed by different researches (Jensen et al., 2004; Fejes et al. 2005; Aggerholm et al., 2008; Wu et al., 2008; Chavarro et al., 2010; Rohrmann et al., 2011; Macdonald, et al., 2013; Lu JC, 2015). The main reason for this decline is thought to be the decrease of SHBG production (Rohrmann et al., 2011; Fejes et al., 2005). Rohrmann et al. (2011) offered that SHBG is probably more strongly affected by abdominal than overall obesity. Levels of fasting glucose and excess monosaccharide consumption rather than elevated insulin levels are potential explanations for declining SHBG (Selva et al., 2007; Peter et al., 2010).

Concomitantly, testosterone levels in obesity can decline due to increased peripheral conversion of androgen to oestrogen which is attributed to higher oestradiol levels and suppression of the HPG axis (Schneider et al., 1979; Kley et al., 1980). However, only some articles show positive associations between adiposity markers and oestradiol levels (Rohrmann et al., 2011; Chavarro et al., 2010; Jensen et al., 2004). In our study, WHtR as well as BMI, BF% and WC were not related to oestradiol.

Majority of the studies, among them ours, have not found correlation between adiposity and gonadotropin levels (Jensen et al., 2004; Fejes et al., 2005; Aggerholm et al., 2008; Chavarro et al., 2010; Lu et al., 2015).

To our knowledge, these study findings were the first to show a relationship between sperm parameters and two adiposity markers (BF% and WHtR) and also the first to show a relationship between serum reproductive hormone levels and WHtR.

7.3.2. The relationships between total testicular volume, reproductive parameters and surrogate measures of adiposity (Paper 3)

7.3.2.1. Total testicular volume, body composition and male reproductive health parameters

The mean and the median of the MPIC study group TTV was 46 ml, which confirms the results of earlier studies (Jørgensen et al., 2002; Erenpreiss et al., 2017).

Parameters characterising height and body composition had a strong relationship with TTV. There is a certain logic to the assumption that taller men have a larger TTV, which has been approved in different studies (Handelsman et al., 1985; Ku et al., 2002; Hart et al., 2015), including ours. In addition, a positive correlation between BMI, WC, WHtR and the TTV was found ($p < 0.001$ for all) (Table 2 in Paper 3).

Regarding reproductive parameters, positive correlations between the TTV and total sperm count, sperm concentration, -morphology, -motility ($p < 0.001$) and semen volume ($p = 0.005$) were found. The men with the largest TTV (TTV ≥ 51 ml) had a mean total sperm count of over four times higher [$254.2 \times$

$10^6/\text{ml}$ (95% CI: 231.5; 279.2)] than the men with the lowest TTV [TTV <37 ml: $56.0 \times 10^6/\text{ml}$ (95% CI: 47.8; 65.5)]. Other researchers have shown similar effects (Sakamoto et al., 2008a; Bahk et al., 2010; Tijani et al., 2014; Ventimiglia et al., 2017). The gonadotropins were negatively ($p < 0.001$) and the testosterone was not correlated to the TTV which have been confirmed earlier (Bujan et al., 1989; Hart et al., 2015). On the other hand, some studies have found a positive correlation between testosterone and the TTV (Sakamoto et al., 2008a; Bahk et al., 2010). In men with a smaller TTV, a comparable testosterone level to those with a larger TTV was achieved, apparently due to an increase in LH levels. As outlined previously, the small testes' Leydig cell function might be better preserved than the seminiferous tubule function (Sakamoto et al., 2008b).

There were no significant differences between the TTV groups when compared for chronic diseases. At the same time, disorders potentially affecting testicular function, especially varicocele, were more prevalent among men with lower testicular volume ($p < 0.001$). The relationship between varicocele and smaller testis volume have shown by Hart et al. (2015). Men with higher testicular volume were older and they consumed significantly more alcohol ($p = 0.006$, $p = 0.023$, respectively). Rastrelli et al., 2013 have also demonstrated a positive relationship between risk behaviour and the TTV (Rastrelli et al., 2013).

7.3.2.2. The relationships between surrogate measures of adiposity and reproductive parameters in different TTV groups (Paper 3)

Clear differences in semen quality between BMI, WC, WHtR and the TTV groups were seen. Importantly, men with the smaller testicles (TTV ≤ 46 ml) were more affected. In the case of a TTV ≤ 46 ml, all three measures (BMI, WC, WHtR) were negatively associated with the total sperm count ($p = 0.003$, $p = 0.008$, $p = 0.001$) and the sperm concentration ($p = 0.005$, $p = 0.015$, $p = 0.002$). Significant differences were found between the first and the fourth quarter. These changes appeared from a BMI ≥ 29.1 , WC ≥ 102 cm and WHtR ≥ 0.56 . Decline in semen quality was more pronounced between WHtR and the TTV (Table 3 in Paper 3). As mentioned above, lower sperm count and -concentration [84.5 (95% CI: 71.2; 100.4), 24.3 (95% CI: 20.6; 28.8), respectively] in the fourth quarter were found compared with men in the first quarter [130.2 (95% CI: 115.2; 147.1), 33.1 (95% CI: 29.4; 37.2), ($p = 0.01$, $p = 0.01$), respectively].

In the case of a TTV > 46 ml, BMI, WC and WHtR were inversely correlated only with the semen volume ($p = 0.085$, $p = 0.03$, $p = 0.01$).

As in the previous study (7.3.1), adiposity parameters were not related to the LH and FSH levels but they were related to the total testosterone levels in both TTV group (for all parameters $p < 0.001$). At the same time, no statistically significant differences were observed when comparing testosterone levels

within the WHtR, WC and BMI quartiles in the TTV groups. With respect to adjusted means of oestradiol, a certain positive association with anthropometric parameters can be seen in the group of TTV > 46 ml, but the post hoc test did not confirm these significant relationships between the groups (excluded from paper).

To our knowledge, the findings of this study are the first to show that in the presence of central adiposity sperm parameters are dissimilar in different TTV groups.

7.3.3. The relationships between metabolic syndrome and reproductive parameters (Paper 4)

Even if the prevalence of MS has been suggested as a possible risk factor that can attribute to infertility, the evidence linking MS with impaired semen parameters is still conflicting. When stratifying men into four groups, MS had no independent effect on major fertility parameters in different subgroups of men (Table 3 in Paper 4). Likewise, no significant differences were found between MS and semen parameters within the BMI categories (Table S1 in Paper 4). Significant differences in semen quality were only obtained between FM and MPIC groups. Conversely, some other studies have found decreased semen quality in men with MS (Lotti et al., 2013; Leisegang et al., 2014; Ventimiglia et al., 2016a; Elsamanoudy et al., 2016). Nevertheless, negative correlation between semen parameters and MS from mentioned studies was obtained from MPIC, infertile men and men from a specific region of South Africa who were older compared to our study groups.

Regarding reproductive hormones, testosterone was strongly correlated to MS ($p < 0.001$) (Table 3 in Paper 4). This negative association persisted even after an adjustment for covariates within the BMI categories, but only between MPIC-MS⁻ and MPIC-MS⁺ groups (post hoc test $p < 0.001$ for BMI 25–29.9). A negative association between testosterone levels and MS have been confirmed by many researches (Laaksonen et al., 2003; Corona et al., 2006; Lotti et al., 2013; Ventimiglia et al., 2016a,b) and a systematic review with meta-analysis showed that MS is significantly and independently associated with an overall lower total testosterone levels (Corona et al., 2011).

In MPIC, LH was negatively correlated to MS ($p=0.048$) (Table 3 in Paper 4). In spite of statistically negative results, the same trend was seen within BMI groups. As already demonstrated before lower LH levels could be explained by larger testicles (Sakamoto et al., 2008a) and MS was positively correlated to the TTV seen in MPIC ($p<0.001$) (Table 2 in Paper 4). FSH as well as oestradiol levels were not related to MS.

7.3.4. The relationships between liver tests and reproductive parameters (Paper 2)

Performed analysis showed that GGT was negatively related to sperm concentration and total sperm count (Table 5) (Table 2 in Paper 2). These significant changes appeared from a GGT >35.5 U/L. A post hoc test demonstrated that men in the 4th quarter had a significantly lower sperm concentration and total sperm count compared with men in the other quarters. The present results revealed, for the first time, an association between semen quality and GGT. Next to these changes ALT was not related to sperm parameters.

Both enzymes, GGT and ALT, were not related to reproductive hormone levels.

The data on associations of liver enzymes and male reproductive health is limited. Only one previous research has been shown that NAFLD non-obese men had significantly poorer sperm quality and lower testosterone and LH levels compared with healthy men without fatty liver (Li Y et al., 2015).

Table 5. Distribution of sperm count and -concentration according to GGT.

	GGT(n=241)					P value
	25th = 15 U/L	50th 21 U/L	75th = 35.5 U/L			
	(n = 61)	(n = 60)	(n = 60)	(n = 60)		
Sperm concentration (×10 ⁶ per mL)						
Adjusted mean (95% CI)	79.6 (64.4; 98.5)	74.1 (60.1; 91.2)	87.8 (71.6; 107.8)	54.0 (43.1; 67.7)	.015	
Total sperm count (× 10 ⁶)						
Adjusted mean (95% CI)	288.6 (230.8; 361.1)	300.4 (241.0; 374.1)	325.1 (262.2; 403.3)	205.4 (161.9; 260.5)	.033	

Sperm parameters were adjusted for BMI, age and alcohol use. ANCOVA with Bonferroni-corrected post-hoc pairwise comparisons was used.

7.4. Pattern of alcohol use and the relationship between alcohol consumption, body composition, liver tests and reproductive parameters

7.4.1. Pattern of alcohol use and the relationship between alcohol intake and body composition

Even if the analysis' results proved no significant difference in the mean amount of alcohol consumed, FM drank less (7.7 units per week) than MPIC (8.7 units per week) (Table 2). At the same time, the alcohol status differed significantly between those groups. Compared to MPIC, more non-drinkers and

less moderate drinkers (< 16 units/week) were found among FM. According to the NIHD, drinking more than 16 units (alcohol) per week for men is defined as heavy drinking (NIHD, 2010). Almost a fifth of our study subjects (FM and MPIC) consumed alcohol ≥ 16 units per week (Table 2). The prevalence of heavy drinking was slightly higher (21%) among men in the general population (Tekkel M, 2017).

After studying the relationship between alcohol intake and body composition in FM (Paper 2), analysis was repeated in MPIC. The results are shown in Table 6. After correction for age, alcohol consumption was positively correlated to BMI, WC and WHtR in both groups. Based on available data, increased energy intake with alcohol use can promote a positive energy balance and weight gain (Lukasiewicz et al., 2005; Niemelä et al., 2017; Alatalo et al., 2008; Downer et al., 2017).

Moreover, we showed that in both groups (FM and MPIC) there were more overweight and obese men than normal-weight men who consumed ≥ 16 units per week ($p=0.035$, $p=0.029$, respectively).

Table 6. The association between alcohol intake and body composition.

	Non alkohol		< 16 units/week		≥ 16 units/week		P	
	FM n=45	MPIC n=230	FM n=162	MPIC n=1318	FM n=43	MPIC n=334	FM	MPIC
BMI (kg/m²) mean \pm SD	24.8 (3.4)	26.0 (4.5)	25.6 (3.5)	26.4 (4.2)	27.1 (4.8)	27.0 (4.4)	0.01	0.016
WC (cm) mean \pm SD	88.7 (9.3)	92.3 (13.2)	90.8 (8.9)	93.7 (11.9)	94.1 (11.5)	96.3 (12.4)	0.022	<0.001
WHtR mean \pm SD	0.49 (0.05)	0.51 (0.71)	0.50 (0.05)	0.52 (0.64)	0.52 (0.06)	0.53 (0.67)	0.013	0.001

FM 10 missing, MPIC 790 missing. The independent t-test was used.

7.4.2. Alcohol consumption in relation to liver tests and reproductive parameters (Paper 2)

A positive correlation between liver tests and drinking was found, but after adjustment for covariates only GGT levels stayed significantly related to alcohol consumption. Positive correlation revealed between non-drinkers [24.2 (95% CI:18.7; 29.7)] and habitual drinkers [36.0 (95% CI: 30.3; 41.6) ($p = 0.012$)] and also between moderate drinkers (28.0 (95% CI: 25.2; 30.8) and habitual drinkers groups ($p= 0.043$). It has been previously confirmed that serum GGT activities in drinkers were higher than that in abstainers (Alatalo et al., 2008; Tynjälä et al., 2012).

Forming three groups based on men's weekly alcohol consumption, we did not find any relationship between drinking and semen quality, which is con-

sistent with previous international cross-sectional study (Jensen et al., 2014). Thereafter, analysis based on alcohol consumption with or without elevated levels of liver enzymes was made. A post-hoc test showed that after adjustment for covariates men who consumed alcohol and had also increased liver enzymes (ALT > 41 U/L and/or GGT > 60 IU) had significantly lower sperm concentration compared with men without elevated liver enzymes but who consumed alcohol (p=0.038). Having found that ALT levels were not related to sperm parameters (7.3.4.) and alcohol consumption, we excluded men with only increased ALT for the next analysis. In our study, elevated GGT levels (> 60 IU) (n=17) were found only among alcohol users irrespective of the amount of alcohol consumed. A post-hoc test revealed that drinkers with elevated GGT had twisely lower total sperm count compared with drinkers without elevated GGT (p=0.010) and with non-drinkers (p=0.029) (Table 7) (Table 3 in Paper 2). Likewise, drinkers with elevated GGT had a significantly lower sperm concentration compared with drinkers without elevated GGT (p=0.009). To the best of our knowledge, this is the first report describing a probable connection between increased GGT, alcohol consumption, and semen quality.

Abnormal liver tests, related to alcohol consumption, were not associated to reproductive hormones. When we repeated analysis but excluded all participants with elevated GGT levels we did not find any relationship between alcohol consumption with elevated levels of ALT, reproductive hormones and sperm parameters.

Table 7. Distribution of sperm count and -concentration according to alcohol use and GGT.

	Liver/ALCO (n=198)			
	No ALCO	ALCO+	ALCO+/GGT	P value
	(n = 35)	(n = 146)	(n = 17)	
Sperm concentration ($\times 10^6$ per mL)				
Adjusted mean (95% CI)	74.1 (56.4; 97.4)	80.9 (71.1; 92.1)	41.8 (27.8; 63.0)	.011
Total sperm count ($\times 10^6$)				
Adjusted mean (95% CI)	309.5 (233.8; 409.3)	302.8 (265.1; 345.9)	155.7 (102.3; 236.8)	.013

ALCO+: drinkers without elevated GGT; ALCO+/GGT: drinkers with elevated GGT. Sperm parameters were adjusted for age and BMI. ANCOVA with Bonferroni-corrected post-hoc pairwise comparisons was used.

8. GENERAL DISCUSSION

The general aim of this work was to investigate the role of adiposity in male infertility to increase knowledge of the reproductive consequences of obesity and obesity-related health and lifestyle conditions.

8.1. Differences in semen quality between FM and MPIC groups

Significantly lower semen quality (except for semen volume) was seen in MPIC compared to FM. Even if we excluded all cases with aspermia, azoospermia, absolute and severe causal factors of infertility forming MPIC study group, twofold lower mean sperm count and -concentration have been found compared to FM. We may hypothesize that one reason for such differences in semen quality can be related to differences in adiposity parameters. We showed that there were almost 10 % more overweight and obese men, 11.5 % more men with WC ≥ 102 cm and 10.1 % more men with WHtR > 0.5 in the MPIC group compared to FM. Leukocytospermia and testicular disorders may also be associated with poor sperm quality. However, we did not find any significant differences between the results of FM and MPIC.

8.2. The relationships between body composition, TTV and semen quality

One of the main findings of the research indicated clearly that body composition plays an important role in male infertility. In both groups (FM and MPIC), central obesity was negatively related to semen quality. In FM, BF% ≥ 23.4 %, WC > 98 cm and WHtR > 0.54 were all negatively associated with lower total sperm count whereas we failed to show associations between BMI and sperm parameters. With further research conducted in MPIC, we found that adiposity was continually associated with reduced semen quality, but the obtained results differed in different subgroups of men. Looking at the anthropometric parameters, it would be logical to expect that WHtR remains unchanged when the TTV increases, but TTV-associated parallel increase of WHtR and WC can be seen. The latter indicates a problematic accumulation of fat in the waist region, seen more in men with a larger TTV. In spite of the results, sperm parameters of centrally obese MPIC (BMI ≥ 29.1 ; WC ≥ 102 cm; WHtR ≥ 0.56) with a TTV of ≤ 46 ml seem to be affected more by weight increase, whereas being overweight may not play such a significant role in men with a TTV > 46 ml. Most of the volume of the testis is made up of seminiferous tubules and as shown by the results, men with larger testicles were able to produce more sperm than men with a smaller TTV. Therefore, men with smaller testicles, even when considered in good health, are more at risk. It is probable that abnormal sperm

quality is reached more quickly by damage related to lifestyle, environmental factors or health problems. On the other hand, fertility problems caused by obesity have probably delayed onset in men with a larger TTV because of better reproductive potential. A positive correlation between testicular volume and weight has already been shown (Handelsman et al., 1982; Spyropoulos et al., 2002; Bahk et al., 2010) while, to our knowledge, there have been no obesity-related infertility studies done in different TTV groups.

Any persisting changes in our health status require time. Together with the TTV, the duration and/or the severity of the adiposity presumably determines, at least partially, a decline in fertility parameters. Even if more than half of the men surveyed (FM and MPIC) had gained the weight during 5 years, normal sperm analyses were still obtained from more than three-quarters of FM and almost half of MPIC. Taking into account the relatively low average age of our subjects, this might be one of the factors that help to explain the obtained results in relation to sperm quality.

In addition to obesity, low weight may reduce sperm quality. Despite the fact that we did not find significant associations between low weight and sperm parameters we showed that distributions of total sperm count and sperm concentration followed inverted J-shaped curve.

8.3. Comparison of different surrogate measures of adiposity

Although there is no perfect marker to describe exact body composition, our results confirmed that, beside BMI, there could be more informative measures of central obesity and adiposity as indicators of male reproductive health. We showed that semen quality was most strongly correlated with WC and BF% in FM. In addition, WHtR seemed to be more predictive surrogate measure of adiposity in the association between TTV and male infertility in MPIC.

While estimating the effect of obesity on male reproductive parameters, BMI is the most commonly used indicator but BMI alone gives no information on the location of body fat (Chan et al., 2003). Taking into account that men are genetically predisposed to develop intra-abdominal obesity, BF%, WC or WHtR might be better markers to determine the influence of adiposity on male reproductive function. Unfortunately, evidence-based cut-off points for BF%, above which there's an increased risk of infertility, does not exist, as it does exist for BMI (Ho-Pham et al., 2011) and WC as a surrogate marker for central obesity ignores the differences in body height. Next to WC, WHtR has the advantage of taking into account the WC as well as height, which avoids some of the problems regarding the measuring of body composition (Ashwell, 2011; Ashwell & Gibson, 2016). The Ashwell Shape Chart figures out that each additional 4 cm of height allows to raise the waistline by 2 cm while keeping WHtR unchanged.

However, even if the weight gain affects fertility potential the negative effect of adiposity are only seen in the highest quartile of WC, WHtR, BF% and BMI, as reported.

8.4. The relationship between metabolic syndrome and semen quality

Abdominal obesity is also an integrated part of MS. Despite our findings, which demonstrated that central adiposity correlates negatively with sperm parameters, we failed to show associations between MS and semen analysis, even in the MPIC group where the prevalence of MS was significantly higher (17.8%) than in the FM group (12.2%). It could be that the clinical manifestation of MS vary between age groups and a combination of certain components of MS and/or the combination of MS with some other factors plays a role. Although we did not find an independent relationship between semen quality and MS within BMI categories, based on our preliminary findings, differences in glycose and insulin levels seem to be interesting in the context of MS and reproductive health. We found significantly higher fasting glucose and insulin levels in MPIC-MS⁺ compared to MPIC-MS⁻. In addition, among MPIC-MS⁺ over 50% of men had higher fasting glucose levels compared to 10.3 % of men among FM-MS⁺. Pitteloud et al., 2005 suggested that Leydig cell steroidogenesis is impaired in insulin-resistant states such as obesity and Leisegang et al. (2014) proposed that hyperinsulinemia is associated with increased seminal insulin concentrations, which may negatively impact the male reproductive function in the case of obesity.

8.5. The relationships between body composition, liver tests and semen quality

Nevertheless, obesity alone may not be a sufficient cause for decline in semen quality. Earlier studies have found that BMI, WC and visceral fat mass are associated with liver enzyme levels (Adams et al., 2008; Verrijken et al., 2010). We confirmed the results of previous reports showing a positive relationship between BMI, WC and liver tests (ALT, GGT) but, more importantly, our analysis revealed that in FM, GGT was negatively related to sperm concentration and total sperm count. GGT is expressed in a number of tissues including many cells within the male reproductive system like the Sertoli and the Leydig cells, the epithelium lining the epididymis, seminal vesicle and vas deferens (Hanigan & Frierson, 1996). As a cell surface glycoprotein GGT is involved in antioxidant defense regulating cellular glutathione (GSH) and cysteine homeostasis. On the other hand prooxidant species (superoxide, H₂O₂, thiyl radicals) are produced during GSH catabolism in selected conditions, particularly in the presence of iron and copper (Whitfield^a, 2001; Paolicchi et

al., 2002; Dominici et al., 2003). Depending on circumstances both anti- and pro-oxidant status could be true. According to studies alcohol consumption has been shown to elevate the indices of iron stores (Whitfield^b et al., 2001; Ioannou et al., 2004; Lieb et al., 2011). Furthermore, it is well known that both iron and alcohol individually cause oxidative stress and that alcoholics have a compromised antioxidant defense system (Singh et al., 2013; Grasselli et al., 2014). Seemingly, the pathological effects of elevated GGT is leading to the production of highly reactive compounds. Potentially harmful effects of GGT, leading to pro-oxidant species formation, could be a possible cause of semen decline. In our study, a significant decline in sperm count and concentration appeared when GGT levels exceeded 35.5 U/L. It has been proposed that elevated serum ALT and GGT levels are independent markers of the activation of systemic inflammation and increased oxidative stress (Yamada et al., 2006). Unlike GGT ALT is not expressed in male reproductive system although it is widely distributed serving as an important contributor to gluconeogenesis and amino acid metabolism (Lindblom et al., 2007; Liu et al., 2014). Due to the differences between GGT and ALT impact in an organism or some other undiscovered reason we failed to show an association between ALT and semen quality.

8.6. Alcohol consumption in relation to body composition, GGT and semen quality

GGT is well known to be related to alcohol use and as predicted, alcohol use was related to GGT also in our study. Troublesome drinking patterns as well as increasing rates of overweight and obesity are widespread problems in Estonia. In 2016, the alcohol consumption was 9.9 litres per capita (aged 15 and over in 100% alcohol) and was higher among men than among women (Orro E et al., 2017). The evidence suggests that alcohol leads to overconsumption of energy, increasing especially consumption of high-fat savoury foods (Schrieke et al., 2015; Downer et al., 2017). Therefore, drinking alcohol can lead to weight gain (Lukasiewicz et al., 2005; Downer et al., 2017). Our results indicate that consumption of alcohol, especially above moderate levels (≥ 16 units/week) can be a risk factor for central obesity that may contribute to the problem of fertility. Interestingly, raise in BMI increases the effect of alcohol consumption on serum liver enzyme activities (Ruhl & Everhart, 2005; Alatalo et al., 2008).

Seeing the interaction between semen parameters and GGT, we were interested in finding out whether the alcohol use could also related to sperm quality. Concentrating on GGT activity, we found a negative relationship between total sperm count, sperm concentration and alcohol consumption in cases with elevated GGT. The decline in semen quality supposedly occurs not due to the amount of alcohol consumed but due to the alcohol use accompanied

by increased GGT activity. Thereby it might be that even moderate drinking could negatively impact semen parameters when the GGT activity increases. As regards influence of alcohol consumption on serum GGT activity aging also appears to play a role and therefore render individuals more susceptible to oxidative stress and ethanol-induced health problems (Tynjälä et al., 2012; Danielsson et al., 2013). Danielsson et al. (2013) showed that in men over 40 years of age, GGT levels appeared to increase with alcohol consumption which were only about half of those found in the age group below 40. The mean age of our participants was 32.3 year which allows us to presume that there may be even bigger decline in total sperm count and sperm concentration as men grow older continuing to drink alcohol.

The relationship between tissue GGT activity and serum GGT activity remains yet unsettled but it has been proposed that subfractionating GGT will be critical in future determinations of the organ-specific mechanisms responsible for increases in serum GGT activity (Bradley R, 2012).

In addition, Rastrelli et al., 2013 have shown a positive relationship between greater alcohol consumption, smoking and the TTV. Our study confirmed a positive association between greater alcohol consumption and a larger TTV. Also, men with larger testicles were more abdominally obese. Besides, animals with a larger TTV are considered more promiscuous (Harcourt et al., 1981; Short, 1997). Taking into account the aforementioned behaviour patterns, it can be assumed that men with a larger TTV may be more prone to lifestyle and diet patterns that favour becoming overweight. It is likely that genetic factors also play a role.

Altogether, the diagnosis of male factor infertility should no longer be regarded only as a fertility concern because semen quality has also been suggested as a strong biomarker of general health (Latif et al., 2017). Figure 4 presents the obtained results.

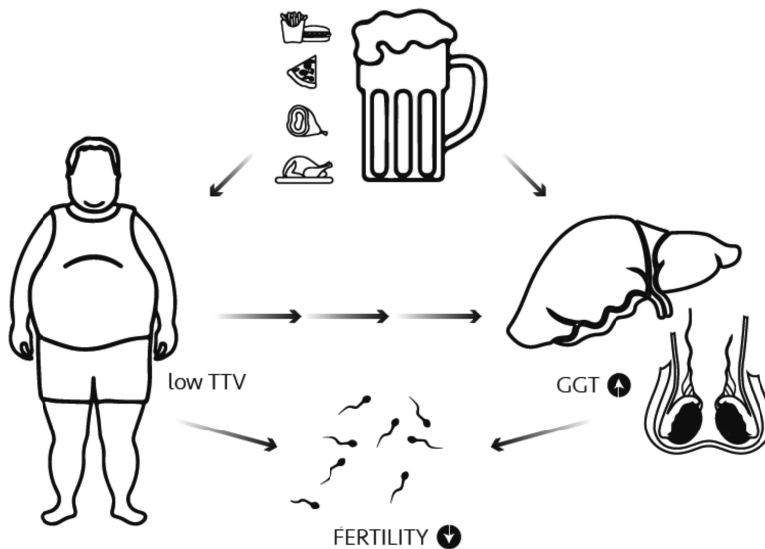


Figure 4. The role of adiposity and potential mechanisms of lifestyle that may affect fertility. Drinking alcohol could lead to weight gain and can be a risk factor for central obesity. Central obesity is negatively correlated to the quality of semen but sperm parameters of centrally obese men with lower total testicular volume (TTV) seem to be affected more by weight increase. Weight increase and alcohol use are positively associated with gamma-glutamyltransferase (GGT) which is expressed in the liver and also in the male reproductive tract. GGT higher activity alone as well as alcohol use accompanied by increased GGT levels are negatively related to semen quality.

8.7. The relationships between body composition, metabolic syndrome, liver tests and reproductive hormones

The entire male reproductive system is controlled by the interaction of hormones. There is compelling evidence that low testosterone precedes the development of obesity and MS (Couillard et al., 2000; Laaksonen et al., 2003). On the other hand, low testosterone is considered to be one of the adverse consequences of adiposity and is a frequent co-factor of MS and its components (Corona et al., 2011; Shin et al., 2012; Leisegang et al., 2014). Therefore the results obtained underline once more that weight gain together (or not) with MS plays a critical role in testosterone decline. Moreover, Lotti et al., 2014 showed that in an age-adjusted logistic ordinal model, insulin levels increased as a function of MS components and showed an inverse correlation with total testosterone (Lotti et al., 2014). As mentioned above, higher fasting insulin levels were found in MPIC-MS+.

We showed that MS and adiposity markers were inversely associated with serum levels of total testosterone and SHBG (in FM) but not with oestradiol. Even if obesity may be associated more with peripheral conversion of androgen to oestrogen, it has been suggested that plasma concentrations of oestradiol can't increase in case of absence of the substrate, testosterone because oestradiol levels are directly related to testosterone levels (Dhindsa et al., 2011). Moreover, Rohrmann et al. (2011) proposed that one reason for differences among studies might be that researchers didn't take into account testosterone and SHBG levels as confounding factors.

Weight gain associated with lower testosterone levels could be combined with increased levels of gonadotropins, but this was not observed. As explained by Teerds K et al. (2011) in general, changes in steroid hormone levels compensate for each other, resulting in normal (unaffected) FSH and LH levels in most overweight and obese subjects (Teerds et al., 2011). The latter approach may also be one possible explanation for our study results. Otherwise, results from the European Male Aging Study supported the hypothesis according to which obesity associated hypothalamic-pituitary dysregulation blunts gonadotropins rise which cannot be compensated by physiological mechanisms (Wu et al., 2008; Tajar et al., 2010). Therefore, it is possible that an increase in body-weight accompanied by other changes in the body (secretion of cytokines and adipokines, sleep apnoea, alterations in hormonal balance, oxidative stress, etc.) prevent an increase in gonadotropins levels. It has been stated that blocked normal physiological reaction to counterbalance testosterone decrease could have a longer term negative effect on the male fertility potential (Fui et al., 2014). In our study we found lower levels of LH seen only in MPIC-MS⁺ compared to MPIC-MS⁻ group. Glucose and insulin metabolism are believed to influence gonadotropins and GnRH neurons can be directly modulated by insulin (Salvi et al., 2006). Based on this, obesity related insulin resistance can result in subnormal secretion of GnRH which in turn diminishes secretion of LH and FSH. Previous studies have shown that low testosterone levels in diabetic men are associated with low serum LH (Dhindsa et al., 2004; Maneesh et al., 2006). Costanzo et al., (2014) hypothesized that impairment of hypothalamic activity appears in diabetic because of the inhibitory effect of hyperglycemia and insulin resistance (Costanzo et al., 2014). Chosich et al., (2017) recently showed that hyperinsulinemia combined with elevated lipids suppresses LH and FSH and neither lipids nor insulin have this effect on their own (Chosich et al., 2017).

GGT and ALT levels were not related to reproductive hormones. Neither did we find associations between alcohol intake and serum sex-hormone levels.

This study had some limitations. A major limitation of our research (in MPIC) can be the lack of SHBG for calculation because it is well known that obesity and MS are associated with the reduction of SHBG levels. Also, our ANCOVA analysis was restricted by missing information regarding lifestyle parameters. Moreover, there is mounting evidence that beside the traditional WHO sperm parameters, the mechanisms inducing changes to sperm molecular

composition are equally important measures of obesity-related male subfertility and may provide additional information, which is lacking in current study.

Despite these limitations, the study had also several strengths. Analysing and comparing data from different subgroups of men (FM and MPIC) certainly gave us a more comprehensive view concerning male reproductive health. In addition, using different surrogate measures of adiposity provided a more thorough understanding of the relationship between obesity and male infertility.

Further perspectives: to continue to find out what is the best body composition marker, showing most clearly the relationships between adiposity and reproductive parameters, to determine the factor or combination of factors of MS that contribute mainly to male infertility and to go forward with research on liver markers in relation to reproductive health.

9. CONCLUSIONS

1. MPIC were more affected by adiposity, especially by abdominal obesity. 60.4 % of MPIC were overweight or obese compared to 51.2% of FM. High risk WC (≥ 102 cm) was found in 26.1% of MPIC compared to 14.6% of FM. High risk WHtR (> 0.5) was found in 57.8% of MPIC compared to 47.7% of FM. Compared to the general population less overweight and obese men were found in the FM group. The prevalence of overweight and obesity was almost comparable between men in the general population and in the MPIC group.
Moreover, 17.8% of MPIC had obesity-associated MS compared to 12.2% of FM of the same age range. At the same time, the prevalence of MS among men in the general population was higher compared with our results.
2. Adiposity, especially abdominal obesity, was associated with decline in semen parameters. The negative effect of adiposity became noticeable in the highest quartile of WC, WHtR, BF% and BMI. Among FM, high WC, WHtR and BF%, were negatively associated with total sperm count. The BF% was also negatively related to semen volume. These changes appeared from a WC > 98 cm, WHtR > 0.54 and BF% ≥ 23.4 %.
In MPIC clear differences in semen quality appeared from a WC ≥ 102 cm, WHtR ≥ 0.56 and BMI ≥ 29.1 and men with smaller testicles (TTV ≤ 46 ml) were more affected by changes in body composition. In the case of a TTV ≤ 46 ml all three measures were negatively associated with the total sperm count and the sperm concentration.
Next to these changes MS had no independent effect on major fertility parameters in different subgroups of men.
3. Adiposity and MS were associated with decline in hormonal parameters. In both groups (FM and MPIC) adiposity markers and MS were negatively correlated to the total testosterone levels. Likewise, negative correlation was found between adiposity markers and SHBG in FM. Adiposity markers were not related to oestradiol and gonadotropin levels. Similarly, FSH as well as oestradiol levels were not related to MS. LH was negatively correlated to MS but only in MPIC.
4. Next to BMI, there were more informative measures of central obesity and adiposity as indicators of male reproductive health. In FM, semen quality was most strongly correlated with WC and BF%. In MPIC, WHtR seemed to be more predictive surrogate measure of adiposity in the association between TTV and male reproductive function.
5. GGT was negatively related to sperm concentration and total sperm count. These changes appeared from a GGT > 35.5 U/L. ALT was not related to sperm parameters. GGT and ALT were not related to reproductive hormones.
6. In both groups (FM and MPIC) alcohol consumption was positively correlated to adiposity parameters (BMI, WC, WHtR). In FM, alcohol

consumption was positively correlated to GGT. Alcohol consumption together with elevated GGT (> 60 U/L) was negatively related to total sperm count and sperm concentration. Abnormal liver tests, related to alcohol consumption, were not associated to reproductive hormones.

10. SUMMARY IN ESTONIAN

Kehakompositsiooni, rasvumise ja rasvumisega seotud eluviisi ning tervisenäitajate seosed mehe reproduktiivfunktsiooniga

Probleemid laste saamisel toovad iga-aastaselt uuringutele üha enam paare. Hinnanguliselt ei suuda aasta jooksul saavutada soovitud rasestumist ligi viisteist protsenti viljakas eas paaridest. Neist pooltel juhtudel osutavad muutused viljakusnäitajates mehega seotud teguritele. Viljakusprobleemidega paaride arvu tõusu põhjuste osas arenenud riikides puudub küll konsensus, kuid oma rolli mängib kindlasti nii ema kui isa ühe kõrgem vanus laste sünnil.

On teada, et viimase sajandi vältel on meeste spermakvaliteet olulisel määral langenud (Carlsen et al., 1992; Levine et al., 2017). Viljakusnäitajate võimaliku halvenemise põhjusena nähakse mitmete teiste tegurite kõrval kiirelt muutunud eluviise. Isegi kui puudub ühtne arusaam langenud viljakusnäitajate seostest eluviisiga, näivad osad faktorid omavat suuremat mõju. Olemasolevad uuringud kinnitavad, et teisenenud liikumis- ja toitumisharjumused ei ole jäänud tagajärgedeta. Paralleelselt mehepoolsete viljakusprobleemide kasvuga on pea kolmekordistunud viljakas eas olevate ülekaaluliste meeste osakaal (Palmer et al., 2012). 2017. aasta Eesti täiskasvanud rahvastiku tervisekäitumise uuringu andmetel oli 59.9 protsenti meie meestest ülekaalulised, kellest rasvunuks hinnati 19.8 protsenti (Tekkel M, 2017). Võttes arvesse kõiki vanusegruppe, avaldub ülekaalulisus varem ning on arvuliselt suurem just meeste hulgas. On uuringuid, mis seostavad rasvumist mehepoolse viljakusvõime langusega (Jensen et al., 2004; Trivison et al., 2007; Hammoud et al., 2008; Hofny et al., 2010; Paasch et al., 2010; Hammiche et al., 2012; Eisenberg et al., 2014; Leisegang et al., 2014; Tsao et al., 2015), kui ka neid, mis ei näe kaalutõusu ja viljakusparameetrite vahel olulisi seoseid (Aggerholm et al., 2008; Li et al., 2009; Duits et al., 2010; Macdonald et al., 2013; Lu et al., 2014). Küll ollakse pea ühiselt nõus, et ülekaal mõjutab hormonaalset tasakaalu. Eelkõige seondub liigne kehakaal meestel testosterooni taseme langusega, mida peetakse omakorda üheks viljakuse languse põhjuseks (Jensen et al., 2004; Fejes et al. 2005; Aggerholm et al., 2008; Wu et al., 2008; Chavarro et al., 2010; Rohrmann et al., 2011; MacDonald et al., 2013; Lu JC, 2014). Kuna metaboolse sündroomi (MS) keskseks komponendiks on vööpiirkonna rasvumine, on antud teemale viljakusuuringutes samuti üha enam tähelepanu pööratud. Uuringuid, mis aga kinnitaksid MS otsest negatiivset mõju spermakvaliteedile, on siiski vähe (Lotti et al., 2013; Leisegang et al., 2014 et al; Ventimiglia et al., 2016b; Elsamanoudy et al., 2016).

Kuigi uuringutes on kehakompositsiooni iseloomustamiseks enim kasutatud kehamassi indeksit (KMI) ei võimalda see hinnata lihasmassi ja rasvkoe osakaalu ning rasvkoe paiknemist kehas. KMI alusel võib normaalse rasvamassi, kuid samas suurema lihasmassiga isik sattuda ülekaaluliste klassi ning aine-

vahetuslikus mõttes rasvunud normkaaluline jääda märkamata (Ruderman et al., 1981; Cabler et al., 2010). Kehakompositsiooni hindamisel KMI-d ainuparameetrina kasutades jäävad toodud eripärad seega tähelepanuta. Kuna terviseriskidega on enam seotud vööpiirkonna rasvahulga tõus, tuleks KMI kõrval võtta arvesse vööümbermõõtu (VÜ). Maailma Tervishoiuorganisatsiooni kohaselt on kaukaasia rassist meestel (sh. eestlastel) $VÜ \geq 102$ cm seotud kõrge ja $VÜ > 94$ cm suurenenud riskidega tervisele (Alberti et al., 2009). Suhteliselt uue, aga käepärase antropomeetrilise näitajana on kasutusele võetud vööümbermõõdu- pikkuse suhe ($VÜ/Pi$), mis võtab vööümbermõõtu hinnates arvesse ka inimese kasvu (Browning et al., 2010). Lubatud referentside kohaselt ei tohiks antud suhe olla suurem kui 0.5 ehk madala riskiga tervisele on vöökoht, mis mõõdetuna on pool või alla poole meie pikkusest.

Meeste viljakust hinnatakse põhiliselt seemnevedeliku analüüsi alusel. Samas on viljakuspotentsiaali hindamise üheks lihtsamaks meetodiks munandi-mahttude (MM) määramine. Kriitilist MM väärtust, mis tagaks adekvaatse reproduktiiv-funktsiooni, pole siiani aga kindlalt määratletud. Uringute põhjal on soovituslik optimaalne munandi maht küllalt laiapiiriline, jäädes vahemikku 14–35 ml (Takahara et al., 1983; Jørgensen et al., 2002; Sakamoto et al., 2008; Stewart et al., 2009; Nieschlag et al., 2010; Andrology Australia, 2014). Eelnevalt on mehe viljakusparameetrite ja MM vahelisi positiivseid seoseid kinnitanud mitmed uuringud (Sakamoto et al., 2008; Bahk et al., 2010; Tijani et al., 2014; Ventimiglia et al., 2016).

Uurimistöö eesmärgid

Uurimistöö üldiseks eesmärgiks oli hinnata kehakompositsiooni, ülekaalu ning ülekaaluga seonduvate tervisenäitajate ja eluviisi seoseid sperma- ning hormoonparameetritega rasedate naiste meestel (RNM) ja viljatute paaride meespartneritel (VPM).

Uurimistöö konkreetSED eesmärgid:

1. Hinnata ülekaalu ja MS levimust RNM ja VPM hulgas.
2. Hinnata erinevate antropomeetriliste näitajate ja MS seoseid sperma parameetritega RNM ja VPM gruppis.
3. Hinnata erinevate antropomeetriliste näitajate ja MS seoseid reproduktiivhormoonidega RNM ja VPM gruppis.
4. Hinnata, milline ülekaaluga seonduv antropomeetriline näitaja toob kõige paremini välja võimalikud seosed kehakompositsiooni, rasvumise, sperma parameetrite ja reproduktiivhormoonide vahel.
5. Hinnata maksaensüümide (GGT, ALT) seoseid viljakusparameetritega RNM gruppis.
6. Hinnata alkoholitarvitamise mõju kehakompositsioonile (RNM, VPM gruppides) ja maksaensüümide tasemele ning seeläbi viljakusparameetritele (RNM gruppis).

Uuritavad ja meetodid

Mitut keskust kaasav (Tartus ja Tallinnas) läbilõikeuring viidi läbi aastatel 2008–2013. RNM uuringugrupi moodustamiseks teavitati uuringust Tartu Ülikooli Kliinikumi ja Lääne-Tallinna Keskhaigla naistekliinikutesse pöördunud 3175 raseda naise meest. Oma nõusoleku uurimistöös osalemiseks andis ligemale kolmandik teavitatutest. Peale esmast andmete läbivaatust kaasati 277 meest, kes olid nõustunud lisaks meditsiinilisele läbivaatusele (antropomeetrilised näitajad ja MM) andma spermaanalüüsi (WHO 2010) ja vereanalüüsi suguhormoonidele (FSH, LH, testosteroon, östradiol, SHBG). Lisaks paluti meestel täita küsimustik, mis sisaldas andmeid nii tervises seisundi kui eluviiside (sh. alkoholitarbimise ja suitsetamise) kohta. Uuringust eemaldati 17 meest. Eemaldamise põhjuseks olid diagnoositud sugutrakti kahjustavad haigused, varasemalt teostatud väikevaagna piirkonna operatsioonid, liiga lühike või pikk abstinentsi periood (WHO 2010) või juhud, kus rasedus oli saavutatud IVF protseduuri abil. Saadud andmete põhjal kaasati antropomeetriliste näitajate ja viljakusparameetrite vahelisi seoseid hindavasse uuringusse 260 meest (vanus 21–57 aastat). Maksaensüümide ja viljakusparameetrite vahelisi seoseid hindavasse uuringusse kaasati 245 meest. Uuringusse võeti mehed, kellel lisaks ülal mainitud tervisenäitajatele oli määratud ka maksaensüümide tase (ALT, GGT). 15 mehel need andmed puudusid. 238 juhul lisandusid andmed vereõhu väärtuste ja üldtervise analüüsides (paastuglukoos, üld-, LDL- ja HDL- kolesterool, triglütseriidid) kohta. Antud mehed kaasati metaboolse sündroomi ja viljakusparameetrite vahelisi seoseid hindavasse uuringusse. Metaboolse sündroomi hindamiseks kasutati Rahvusvahelise Kolesterooli Õppeprogrammi Täiskasvanute III Ravipaneeli (NCEP ATP III) kriteeriume.

VPM uuringugrupp moodustati TÜK meestekliinikusse uuringutele pöördunud patsientide baasil. Uuringusse kaasamise kriteeriumiks oli varasem teadaolev paariviljatuse rohkem kui ühe-aastase perioodi jooksul. Uuritavatel teostati meditsiiniline läbivaatus, seemnevedeliku analüüs (WHO 2010) ning võeti vereanalüüsid suguhormoonidele (FSH, LH, testosteroon, östradiol) ja üldtervise näitajatele (vt. ülal). Lisaks paluti täita terviseküsimustik. Uuringust eemaldati alla ühe-aastase paariviljatuse anamneesiga ning aspermia või azoospermia mehed ning need, kellel oli diagnoositud kindel viljatusega seondud haigus (sugutrakti arenguhäired, geneetilised põhjused, jm), liiga lühike või pikk abstinentsi periood, kes olid üle 50 aasta vanad või kellel puudusid andmed antropomeetriliste- hormoon- ja/või üldtervise näitajate kohta. Saadud andmed võimaldasid kaasata MM, antropomeetriliste näitajate ja viljakusparameetrite vahelisi seoseid hindavasse uuringusse 2672 meest ning metaboolset sündroomi ja viljakusparameetrite vahelisi seoseid hindavasse uuringusse 2642 meest.

Mõlema grupi puhul (RNM ja VPM) kasutati kehakompositsiooni hindamisel järgmisi parameetreid: KMI, VÜ, VÜ/Pi. RNM grupi puhul lisandus keha rasvaprotsent (RP). Meeste alkoholitarvitamise harjumusi hinnati AUDIT testi alusel (Babor TF et. al, 2001). Mehed jagati alkoholitarbimise kogustest

lähtuvalt kolme gruppi arvestades üheks alkoholiühikuks 10 g puhast alkoholi (mitte-tarbijad, < 16 ühiku/ nädalas, ≥ 16 ühiku/ nädalas).

Statistilises analüüsis kasutati tarkvarapaketti SPSS Windows 20.0 (SPSS Inc. Chicago, IL). Lubatud statistilise vea piiriks valiti 5% ($p < 0.05$).

Uuringus osalemine oli vabatahtlik. Uuring oli eelnevalt heaks kiidetud Tartu Ülikooli Inimuuringute Eetikakomitee poolt (eetika komitee protokoll No 152/4, 2006 ja protokoll No 188/M-16, 2009).

Uurimistöö tulemused ja järeldused

Meie uurimistöö täiendas seniseid teadmisi ülekaalu ning ülekaaluga seonduvate tervisenäitajate ja eluviisi mõjust mehe reproduktiivfunktsioonile.

1. Võrreldes RNM grupiga avaldusid ülekaal ja rasvumine, eelkõige vööpiirkonna rasvumine, suuremal määral VPM grupis. VPM hulgas oli ülekaalulisi 60.4%, kellest rasvunuks võis lugeda 19.9 %. Samas oli RNM grupis ülekaalulisi 51.2 %, kellest rasvunuid 14.7%. VPM grupis esines kõrge riski VÜ (≥ 102 cm) 26.1%-l ja kõrge riski VÜ/Pi (> 0.5) 57.8%-l. RNM grupis olid vastavad näitajad 14.6% ja 47.7%. Ülekaalu ja rasvumise esinemissagedus VPM grupis oli võrreldav mehi iseloomustavate näitudega üldpopulatsioonis. Ülekaalu ja rasvumise esinemissagedus RNM grupis oli aga üldpopulatsiooniga võrreldes madalam. MS esinemissagedus VPM grupis oli 17.8 % ja RNM grupis 12.2 %. Saadud tulemused olid madalamad võrreldes MS esinemissagedusega üldpopulatsiooni meeste hulgas.
2. Vööpiirkonna rasvumine seonduv uuritavatel sperma parameetrite langusega. RNM grupis esines oluline negatiivne seos antropomeetriliste näitajate (VÜ, RP, VÜ/Pi) ja spermatooside koguarvu ning RP ja sperma mahu vahel. Samas ilmnis kaalutõusu ja vööpiirkonna rasvumise negatiivne mõju sperma parameetritele alles siis kui $RP \geq 23.4\%$, $VÜ > 98$ cm ja $VÜ/Pi > 0.54$.

VPM grupi andmeid analüüsides leidsime positiivse seose antropomeetriliste- (pikkus, KMI, VÜ, VÜ/Pi) kui ka reproduktiivse funktsiooni parameetrite (va. testosterooni tase) ja MM vahel. Uuritavate keskmine bilateraalne MM oli 46 ml. Eelduste kohaselt võiks MM kasvades VÜ/Pi suhe jääda muutumatuks, kuid nii mõõdetud VÜ kui VÜ/Pi suhe viitavad suurema MMga meeste puhul probleemsele rasva kogunemisele vöö piirkonda. MM tõusuga paralleelselt suurenes ka RP. VPM grupis saadi negatiivne seos antropomeetriliste näitajate (KMI, VÜ, VÜ/Pi), MM, sperma mahu, spermatooside koguarvu ja -kontsentratsiooni vahel. Olulise tulemusena ilmnis, et kaalutõusust ja vööpiirkonna rasvumisest olid enam mõjutatud mehed MMga ≤ 46 ml. Võrreldavalt RNM grupiga ilmnisid siingi muutused alles tugevalt väljendunud kehakompositsiooni muutuste ja kaalutõusu puhul: $KMI \geq 29.1$, $VÜ \geq 102$ cm, $VÜ/Pi \geq 0.56$. Arvestades saadud tulemusi oleks kehakaalu tõusust tulenevaid viljakust mõjutavaid riske kaaludes vaja kõrvuti kehakompositsiooniga hinnata ka MM.

MS ja sperma näitajate vahel seoseid ei leitud (RNM, VPM grupis).

3. Kehakompositsiooni parameetrid seondusid negatiivselt testosterooni (RNM, VPM grupis) ja SHBG tasemega (RNM grupis), kuid mitte teiste suguhormoonidega (RNM, VPM grupis).
Kolm kehakompositsiooni parameetrit seondusid negatiivselt testosterooni tasemega, kuid sarnaselt RNM grupiga, puudus seos teiste suguhormoonidega. MS esinemise korral leiti negatiivne seos testosterooni tasemega (RNM, VPM grupis). VPM grupis leiti negatiivne seos ka MS ja LH taseme vahel.
4. Kehakaalu ja vööpiirkonna rasvumist iseloomustavate antropomeetriline näitajate puhul täheldati, et KMI kõrval leidub teisi parameetreid, mis toovad selgemini esile seosed kehakompositsiooni, rasvumise, sperma parameetrite ja reproduktiiv hormoonide vahel. RNM grupis ilmnemad kõige tugevamad seosed viljakusparameetrite ning VÜ ja RP vahel. VPM grupis saadi kõige tugevam negatiivne seos viljakusparameetrite ja VÜ/Pi vahel.
5. Uuringu tulemused kinnitasid varasemat teadmist positiivsest seosest kaalutõusu ja maksaensüümide (ALT, GGT) taseme vahel. Teadaolevalt näitasime aga esmakordselt seoseid viljakuse languse ja GGT taseme vahel (RNM grupil). Meie uuringus seondus GGT taseme tõus > 35.5 U/L spermatoosoidide koguarvu ja -kontsentratsiooni olulise langusega. ALT tõus sperma näitajatele mõju ei avaldanud. Samuti ei leidnud me seoseid ALT, GGT ja suguhormoonide taseme vahel.
6. Lähtuvalt kogustest oli alkoholitarbimine mõlemas grupis (RNM, VPM) seotud kaalutõusu ja vööpiirkonna rasvumisega (KMI, VÜ ja VÜ/Pi alusel). Lisaks saime positiivse seose alkoholitarbimise ja bilateraalse MM kui alkoholitarbimise ja MS esinemise vahel.

RNM grupilt saadud andmete kohaselt seondus alkoholitarbimine positiivselt GGT tõusuga, kuid analüüsi tulemuste põhjal puudus alkoholitarbimisel eraldiseisvalt mõju spermanäitajatele. GGT taseme tõus (> 60 U/L) koos alkoholitarvitamisega oli aga seotud spermatoosoidide koguarvu ning – kontsentratsiooni langusega. Hormoonnäitajate, alkoholitarbimise ja maksaensüümide tõusu vahel seoseid ei avaldunud.

ACKNOWLEDGEMENTS

I would like to thank with my deepest respect:

- My supervisor, Associate Professor Margus Punab, for introducing me the field of andrology and male reproduction as the teacher. I appreciate greatly his highly effectual and pertinent feedback to my work and his support and encouragement;
- Professor Vallo Volke and to Associate Professor Kristiina Rull for critically reviewing and improving my thesis;
- Professor Sonia Brichard for introducing me the field of obesity and nutrition as the teacher;
- Mrs. Heti Pissarev and Mrs. Eha Nurk for excellent statistical support;
- Mr. Marko Levin for original illustrations on the manuscript;
- Mr. Paavo Kuiv for technical support;
- Our nurse, Triina Karlson, helping me with study patients;
- All the colleagues from the Andrology Centre for technical advices, for their supportive attitude and for always providing a friendly working atmosphere;
- All the patients who spent their time and agreed to participate in our study;
- My daughter Eeva for her thorough linguistic revision of texts of original publications and the manuscript;
- All my family, particularly my husband Tiit, for their support, understanding and patience during all those years;

This study was funded by the European Regional Development Fund, project HAPPY PREGNANCY, no. 3.2.0701.12-004, and by the Estonian Research Council: grant no. PUT181.

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PUBLICATIONS

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2. Ausmees, K., Ehala-Aleksejev, K., Punab, M. (2008). Associations between body mass index and lower urinary tract symptoms. *International Journal of Andrology: 5th European Congress of Andrology*. Wiley InterScience, 33–33.

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7. Ehala-Aleksejev, K., Punab, (2016). Serum hepatic enzyme activity in relation to semen quality and serum reproductive hormone levels among Estonian fertile Men. *Andrology*, 4, 152–159
8. Ehala-Aleksejev, K., Punab, M. (2017). Relationships between total testicular volume, reproductive parameters and surrogate measures of adiposity in men presenting for couple's infertility. *Andrologia*, e12952
9. Ehala-Aleksejev, K., Punab, (2018). The effect of metabolic syndrome on male reproductive health: a cross-sectional study in a group of fertile men and male partners of infertile couples. *PLoS One*, 13(3):e0194395

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DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

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