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# Reproductive function in middle-aged males: Associations with prostate, lifestyle and couple infertility status

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# LIST OF ORIGINAL PUBLICATIONS

I Reproductive function in middle-aged males: healthy men versus male partners of infertile couples. Kristo Ausmees, Reet Mändar, Paul Korrovits, Mihhail Žarkovski, Gennadi Timberg, Margus Punab. Andrologia 2014; 46(2): 118–125.

II Semen quality and associated reproductive indicators in middle-aged males: the role of non-malignant prostate conditions and genital tract inflammation. Kristo Ausmees, Paul Korrovits, Gennadi Timberg, Margus Punab, Reet Mändar. World Journal of Urology 2013; 31(6): 1411–1425.

III Decline of seminal parameters in middle-aged males is associated with lower urinary tract symptoms, prostate enlargement and bladder outlet obstruction. Kristo Ausmees, Paul Korrovits, Gennadi Timberg, Margus Punab, Reet Mändar. Brazilian International Journal of Urology 2013; 39: 727–740.

IV Semen quality in middle-aged males: associations with prostate-specific antigen and age-related prostate conditions. Kristo Ausmees, Paul Korrovits, Gennadi Timberg, Triin Erm, Margus Punab, Reet Mändar. Hum Fertil (Camb). 2014 Feb 24. [Epub ahead of print]. DOI: 10.3109/14647273.2014.881563.

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Paper I: study design, clinical evaluation, data analysis, writing the paper

Paper II: study design, clinical and ultrasound evaluation, data analysis, writing the paper

Paper III: study design, clinical and ultrasound evaluation, data analysis, writing the paper

Paper IV: study design, clinical evaluation, ultrasound and ultrasound-guided prostate biopsies, data analysis, writing the paper

# ABBREVIATIONS

| | |
|---|---|
| BMI | body mass index |
| BPH | benign prostatic hyperplasia |
| CBP | chronic bacterial prostatitis |
| CI | confidence interval |
| CONC | sperm concentration |
| CP/CPPS | chronic non-bacterial prostatitis/chronic pelvic pain syndrome |
| FSH | follicle-stimulating hormone |
| GnRH | gonadotropin releasing hormone |
| HIV | human immunodeficiency virus |
| HPT | hypothalamus–pituitary–testicular axis |
| $E_2$ | oestradiol |
| IL-6 | interleukin-6 |
| I-PSS | International Prostate Symptom Score |
| IQR | interquartile range (25th–75th percentile) |
| LH | luteinizing hormone |
| LUTS | lower urinary tract symptoms |
| METS | metabolic syndrome |
| MOTIL | sperm motility |
| NIH-CPSI | National Institutes of Health Chronic Prostatitis Symptom Index |
| PCa | prostate cancer |
| PIN | prostatic intraepithelial neoplasia |
| PSA | prostate-specific antigen |
| PVR | post-voided residual urine |
| ROCC | receiver operating characteristic curve |
| ROS | reactive oxidative species |
| SHBG | sex-hormone binding globulin |
| SEVOL | semen volume |
| TGCT | testicular germ cell tumour |
| TPV | total prostate volume |
| TSC | total sperm count |
| WBC | white blood cells |
| WHO | World Health Organization |
| QMAX | maximum urinary flow |

# INTRODUCTION

In recent years, semen quality and fertility in aging male have received increasing attention for several reasons. First, there is a trend in developed countries toward higher paternal (and maternal) age, predominantly due to socioeconomic factors. Family planning often begins after the pursuit of education and establishment of professional career, resulting in higher paternal age. Moreover, divorce, remarriage, and the wish to be a father of child(ren) in a new partnership are increasing trends. Secondly, recent improvements in assisted reproduction technology, particularly in vitro fertilisation and intracytoplasmic sperm injection, have enabled males and couples who were previously considered infertile to produce genetic offspring (Jungwirth *et al.*, 2013).

At the same time, the accumulation of several factors and conditions occurs along with aging that may affect male reproductive function. There are some reports on the impacts of paternal occupation on seminal function (Kenkel *et al.,* 2001; Magnusdottir *et al*., 2005). Also, vascular, hormonal, metabolic and malignant diseases, injuries, lifestyle-related conditions and accumulation of toxic substances may alter reproductive function.

Thus, male aging is a multifactorial process that causes changes in different reproductive organs, including the prostate. The prostate, an accessory gland of the male reproductive system, is directly related to male reproductive, sexual, and ejaculatory functions (McVary, 2006; Hellstrom *et al.,* 2009, Gacci *et al*., 2011). Although benign prostatic hyperplasia (BPH), a progressive condition characterized by prostate enlargement and lower urinary tract symptoms (LUTS), is uncommon before 40 years of age, roughly 50% of men develop BPH-related symptoms by 50 years. The incidence of BPH increases by 10% per decade and reaches 80% by 80 years of age (Oelke *et al.,* 2013). In addition, recent studies have described the risks of prostatitis, male accessory gland infections and prostate cancer in middle-aged infertile males (Rolf *et al*., 2002; Ruhayel *et al*., 2010; Walsh *et al*., 2010; Ng *et al*., 2004). However, little is known about the associations between reproductive function and prostate pathologies in males over 45 years. Moreover, middle-aged males have been generally underrepresented in clinical studies and data on reproductive function in healthy and infertile males over 45 years are limited (Stewart, Kim, 2011; Sartorius, Nieschlag, 2010). Also, only few published studies have examined hormonal status or potential confounders in men over 45 years of age (Merino *et al*., 1995; Arai *et al*., 1998; Pasquallato *et al.*, 2005; Hellstrom *et al.,* 2006).

Therefore, the main aims of this work were to specify reproductive function in middle-aged males (age 45 to 69 years), and to find out possible influences of different prostate conditions and lifestyle-related factors on male reproduction.

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

# LITERATURE REVIEW

## 1. Male reproductive tract

### 1.1. Anatomy

The male reproductive system consists of primary reproductive organs, testicles, and the secondary reproductive organs, the scrotum, urethra, prostate gland, penis, bilateral epididymides, ductus deferenses, seminal vesicles, and bulbourethral glands (Figure 1). 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. The testicle is divided into 250 to 300 lobules, each containing 1–3 seminiferous tubules. Sertoli cells that support sperm production in adult male are located on the basal membrane of tubules.

After spermatogenesis, newly formed sperm cells are transported from the testicles to the epididymis on the external surface of each testicle, and then through the ductus deferens into the pelvic cavity. The ductus deferens joins the ducts of the seminal vesicles, the glands located next to the ampulla of the ductus deferens, and forms the ejaculatory duct, surrounded by the walnut-size prostate gland, and empties into the urethra. The urethra exits from the pelvis and passes through the penis to the outside of the body (Seeley *et al*., 1999).

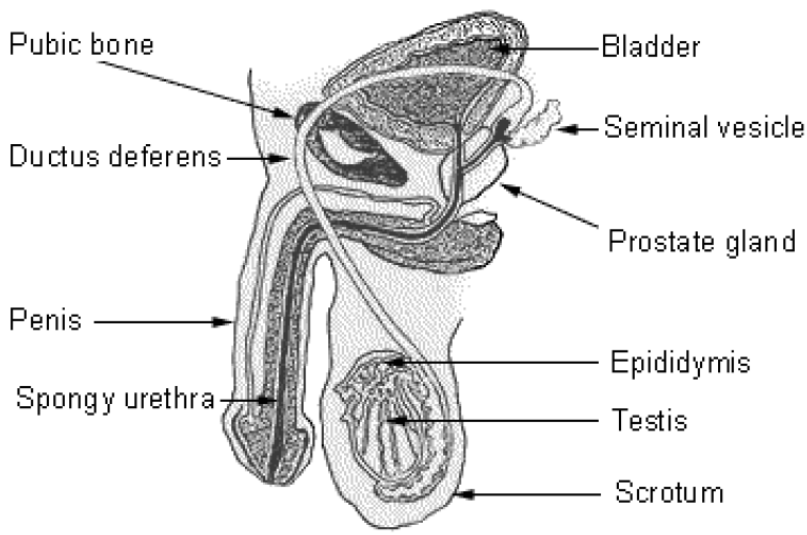


**Figure 1.** Anatomy of male reproductive system.

### 1.2. Physiology

The male reproductive system depends on hormonal, sperm-producing, and neural mechanisms (Seeley *et al*., 1999; Nieschlag, Behre, 2010). Neural mechanisms are primarily involved in controlling ejaculatory and urinary function, and expressing sexual acts and behaviours. Hormonal mechanisms that influence the male reproductive system involve the hypothalamus and pituitary gland in the central nerve system and the testicles. The main hormonal functions are described in chapter 2.2.

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 (Nieschlag, Behre, 2010). Sperm cells from testicles and secretions from reproductive glands form semen as the main product of the male reproductive tract. The seminal vesicles produce about 60%, the prostate gland approximately 30%, and the testicles, epididymides and bulbourethral glands account for approximately 10% of seminal fluid (Seeley *et al*., 1999; Nieschlag, Behre, 2010). The main associations of hormonal mechanisms and production of semen are described in Figure 2.

## 2. Male reproductive function

### 2.1. Semen quality

Semen analysis is the basis of assessments of male reproductive function. Ejaculate analysis and lower reference limits for semen parameters have been standardised by the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen (WHO, 2011) and are described in Table 1.

**Table 1.** Lower reference limits (5th centiles and their 95% Confidence Intervals, CIs) for main semen characteristics (WHO, 2011).

| **Parameter** | **Lower reference limit** |
|---|---|
| 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) |

According to the WHO (2011), oligozoospermia is characterised by a total sperm number less than $39 \times 10^6$ or sperm concentration less than $15 \times 10^6$/mL, asthenozoospermia with less than 32% progressively motile spermatozoa, and teratozoospermia with normal morphological forms of sperms below 4% in ejaculate (WHO, 2011; Jungwirth *et al*., 2013).

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 of semen analyses are abnormal in at least two tests, further andrological investigations are indicated (Jungwirth *et al*., 2013).

Semen has two major quantifiable attributes, total number of spermatozoa and fluid volume. The number of spermatozoa reflects sperm production by the testicles and the patency of the post-testicular duct system, whereas the fluid volume contributes to the function of accessory glands and their secretory activities (WHO, 2011). During ejaculation, semen is produced from a concentrated suspension of spermatozoa, stored in paired epididymides, mixed and diluted by fluid secretions from the accessory sex organs. Semen quality depends on 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 collection of complete/ incomplete semen sample and the size of the testicles (WHO, 2011).

## 2.2. Reproductive hormones

The major male hormone, testosterone, is secreted in the testicles (Figure 2) and is responsible for the differentiation of male genitals and reproductive system, spermatogenesis and secondary sexual characteristics. Testosterone may exert its effects directly, or after its metabolism to dihydrotestosterone or estradiol ($E_2$).

Testicular function is regulated by luteinizing hormone (LH) activating the interstitial Leydig cells in the testicles to produce testosterone, and by follicle-stimulating hormone (FSH) promoting spermatogenesis via testicular Sertoli cells. Release and production of the LH and FSH in the hypophysis are controlled by gonadotropin releasing hormone (GnRH) from the hypothalamus.

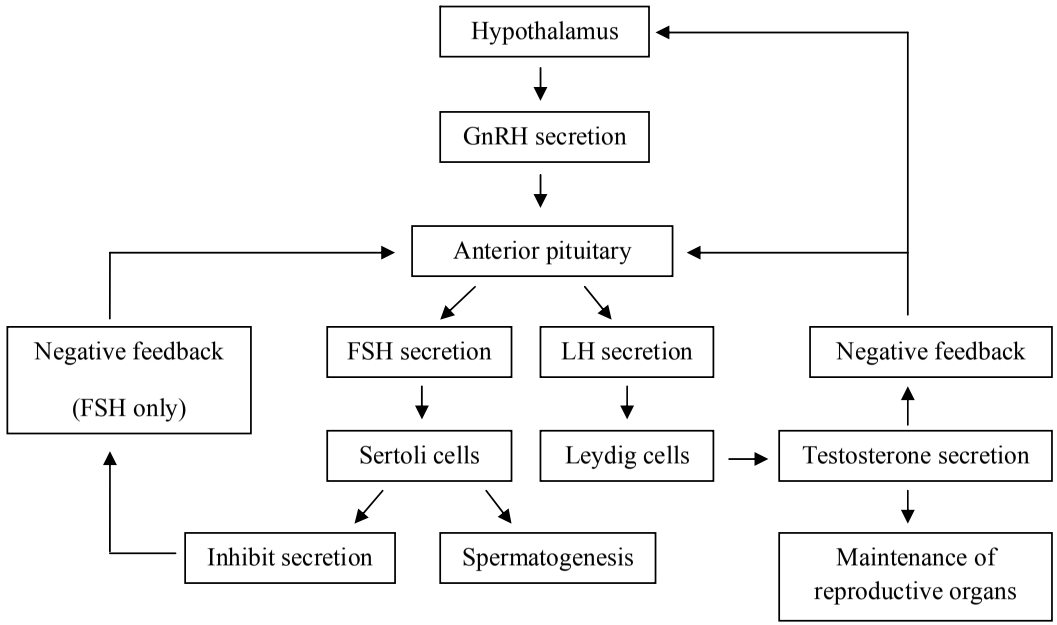


**Figure 2.** Physiology of spermatogenesis and hormonal function in male reproductive tract.

Hormonal alterations of the hypothalamic-pituitary-testicular (HPT) axis are well-accepted, although they are not common causes of male infertility. Even though serum gonadotropin levels could be variable because of pulsatile secretion, a single measurement is usually sufficient to determine a patient's clinical endocrine status. An accurate endocrine evaluation should be performed if there is: (1) an abnormal semen analysis, especially if the sperm concentration is less than 10 x $10^6$/ml; (2) impaired sexual function; or (3) other clinical findings suggestive of a specific endocrinopathy (Jarow *et al*., 2011; Jungwirth *et al*., 2013).

### 2.3. Associations between reproductive function and prostate

A healthy human prostate is slightly larger than a walnut. The mean weight of a "normal" prostate from an adult male is approximately 15 to 25 grams. It surrounds the urethra just below the urinary bladder and can be felt during a digital rectal examination. The prostate can be divided in two ways: by lobes (anterior, posterior, lateral and median) or by zones (Figure 3). It does not have a capsule; rather, it is surrounded by an integral fibromuscular tissue and sheathed by the pelvic floor muscles, which contract during ejaculation. The prostate plays a role in male seminal function and the gland is the main source of prostate-specific antigen (PSA), zinc, citric acid and prostatic acid phosphatase (Elzanaty *et al*., 2002; Sampson *et al*., 2007; Nieschlag, Behre, 2010).

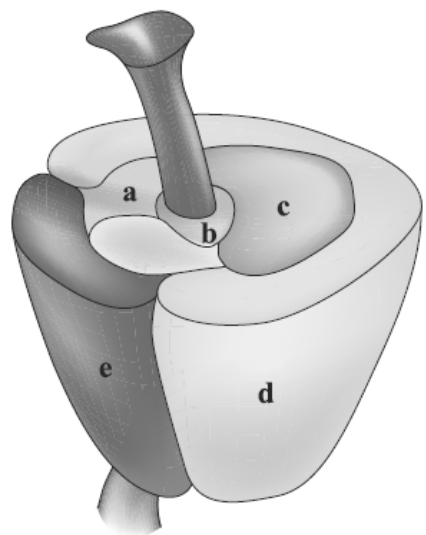


**Figure 3.** Anatomical position of prostate zones (a, transitional zone; b, periurethral zone; c, central zone; d, peripherial zone; e, fibromuscular zone).

Prostatic fluid is expelled in the first ejaculate fraction with most of the spermatozoa. Compared to the few spermatozoa that are expelled with the seminal vesicular fluid, those expelled in prostatic fluid have better motility and survive longer. Associations between reproductive function and different prostate conditions have been discussed in chapter 3.2.1.

# 3. Age-related changes in male organism

Aging commonly is a normal physiological process that involves morphological and functional changes within organs and tissues, accompanied by impairment of certain functions and increased susceptibility to age-related diseases. Although there are no specific aging diseases, the incidence of chronic diseases increases with age (Nieschlag, Behre, 2010).

Known alterations in the aging male involve several organ and regulatory systems, such as the endocrinological and cardiovascular systems, lipid and bone metabolism, and genitourinary tract function. Reductions of growth hormone, dehydroepiandrosterone, dehydroepiandrosterone sulphate, melatonin, thyroid-stimulating hormone, triiodothyronine, thyroxine, and hyperinsulinemia also occur in older males (Nieschlag, Behre, 2010).

## 3.1. Impact of aging on male reproductive function

Like other processes in the human body, aging of the male reproductive tract is accompanied by multifactorial changes at the molecular, cellular and regulatory levels (Kirkwood, 2005). These changes affect various levels of the HPT axis, leading to changes in circulating androgen levels and later effects in androgenic organs. Although the roles of gonadotropins and androgens on spermatogenesis are well-established, the impacts of a secondary decrease in androgen levels on spermatogenesis and fertility are still unclear (Sartorius, Nieschlag, 2010). Also, the proportion of sex-hormone binding globulin (SHBG) increases, whereas bioactive free testosterone decreases, in older male (Nieschlag, Behre, 2010).

Evidence from clinical studies suggests that age is associated with diminished semen volume, sperm motility, and sperm morphology, but sperm concentration is little affected by age (Kidd *et al*., 2001; Kuhnert, Nieschlag, 2004; Sartorius, Nieschlag, 2010; Harris *et al*., 2011). Methodologically stronger studies have shown that semen volume decreases 3%–22%, sperm motility 3%–37%, and percent of normal sperms 4%–18%, comparing 30- to 50-year-old men (Kidd et *al*., 2001). Considering the WHO reference ranges (WHO, 1999), a study by Ng *et al.* (2004) showed that semen volume, sperm concentration and total sperm count were subnormal in 20%, 22% and 22%, respectively, of men at 52 to 79 years of age. Among the subjects in younger group (age <52 years), comparable figures were 20%, 6.5% and 6.9%, respectively. The proportion of men with completely normal semen analysis (semen volume, sperm concentration and total sperm count above reference ranges) was

significantly lower among older men (30%) compared to younger men (72%). Moreover, Dunson *et al.* (2004) showed that seminal parameters start to decline after 35 years of age, resulting in reduced fertility and diminished fecundity a few years later (Kuhnert, Nieschlag, 2004).

In infertile couples, 20–30% of men have low testosterone level (Lombardo *et al*., 2005). The frequency of sexual activity per month for men decreases, on average, by 50% between 50 and 60 years of age, and another one to two times after age 60 years (Araujo *et al*., 2004). Although reduced male sexual activity is more often related to psychological changes, the age-related decrease of sexuality may be an indirect reason for couple's infertility (Shindel *et al*., 2008). However, the lack of correlation between semen parameters and serum testosterone levels suggests that changes in spermatogenesis are not due to a loss of testicular steroidogenic potential.

Testicle volume, a rough indicator of Sertoli cell number and spermatogenic potential (Harris *et al*., 2011), remains constant throughout life. Excluding men with diseases known to reduce testicular size, age reduces testicular volume only in the eighth decade of life or later. In healthy 80-year-old men, testicle volume was, on average, 20–31% lower compared to males under 40 years old (Handelsman, Staraj, 1985; Mahmoud *et al*., 2003).

Aged testicles have reduced number of Leydig and germ cells, thickening of the basal membrane and defective vascularization of testicular parenchyma, including Sertoli cells (Dakouane *et al*., 2005). These changes might impair spermatogenesis and diminish feedback from the testicles to the pituitary gland, resulting in elevated serum FSH and LH levels. Higher serum FSH levels has been correlated with decreased sperm concentration, decreased sperm motility, and a decreased percentages of sperm with normal morphology, whereas higher serum LH level was correlated with decreased sperm concentration (Sartorius, Nieschlag, 2010).

There are no longitudinal studies evaluating the effect of aging on various aspects of reproductive function, and guidelines for evaluating older fathers have not been developed (Stewart, Kim, 2011). Men older than 50 years have been underrepresented in clinical studies, and limited statistical power has prevented the determination of the shape of the relationship between age and semen quality. The main changes in the aging male reproductive tract are presented in Figure 4.

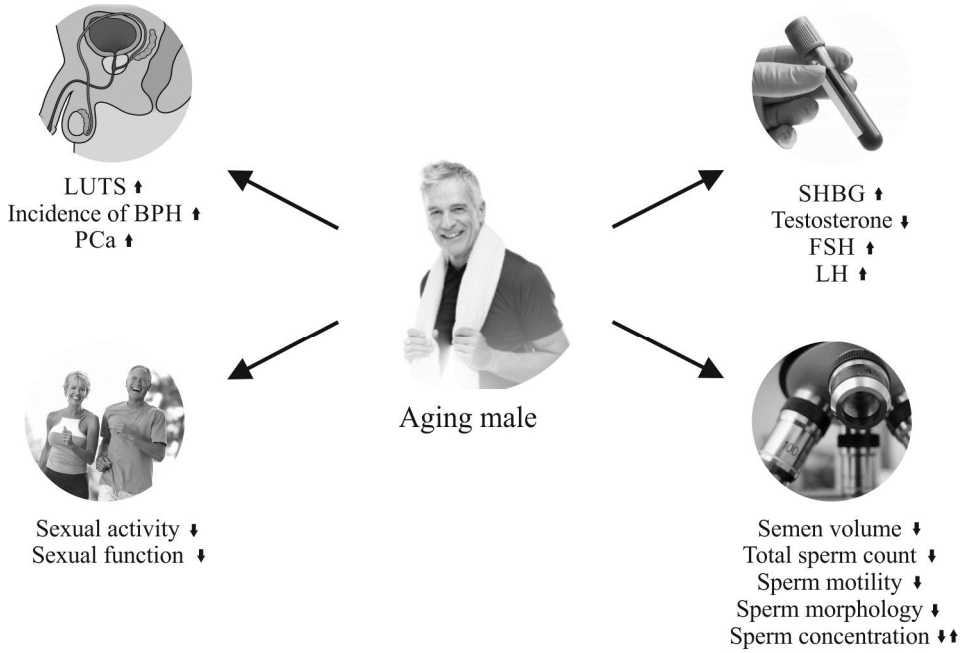


**Figure 4.** Main alterations of the aging male reproductive tract.

### 3.2. Causes for alterations in the aging male reproductive tract

Age-dependent alterations of semen parameters may have several causes. In addition to age *per se*, factors such like injuries, accumulation of toxic substances, vascular, hormonal and metabolic diseases, and anatomical or physiological conditions may be responsible for deterioration of semen parameters.

#### 3.2.1. Prostate pathologies

Previous reports have revealed changes in the prostate of the aging male, such as smooth muscle atrophy and a decrease in protein and water content, that may contribute to decreased semen volume and sperm motility (Kuhnert, Nieschlag, 2004).

Well-known pathological conditions of prostate include prostate inflammation, BPH and prostate cancer (PCa). These age-dependent prostate alterations are detectable histologically in 50% of 50-year-old and 80% of 80-year-old men (Hermann *et al*., 2000). PCa, the most common non-cutaneous malignancy in men of Western countries, is strongly associated with age. BPH, prostatic intraepithelial neoplasia (PIN) as the premalignant and -invasive stage of prostatic adenocarcinoma and PCa have been identified as sequelae of chronic intraprostatic inflammation (Nelson *et al*., 2004; Kramer, Marberger, 2006; Heidenreich *et al*., 2013).

### 3.2.1.1. Prostate inflammation

Prostate inflammation (prostatitis) is a common condition being one of the most frequent urologic diseases in men (Engeler *et al*., 2006). According to National Institute of Health classification (Krieger *et al*., 1999), prostatitis occurs in four forms: 1) acute bacterial prostatitis, 2) chronic bacterial prostatitis (CBP), 3) chronic nonbacterial prostatitis/chronic pelvic pain syndrome (CP/CPPS) and 4) asymptomatic prostatitis. Studies of semen quality have been carried out mainly in younger males, and therefore associations between the prostate inflammation and reproductive function in aging males are underinvestigated. Also, one of the most important drawback of the topic is lack of age-matched controls (Engeler *et al*., 2006).

Male infertility and altered seminal function may be one of the most serious consequences of CBP and CP. Potential mechanisms include obstruction of male genital tract, alterations in the prostate's biochemical environment, formation of reactive oxidative species (ROS), and leukocyte infiltration. Leukocytospermia and ROS have been associated with infertility and alterations in seminal parameters, such as sperm count, motility and morphology (Schoor, 2002; Menkveld *et al*., 2003; Agarwal *et al*., 2006; Shi *et al*., 2012). Although unconfirmed, seminal leukocytes, produced predominantly in prostatic fluid, may provide the connection between ROS generation and impaired semen parameters (Schoor, 2002; Shi *et al*., 2012). There are several reasons why spermatozoa are more vulnerable to ROS than other cells. First, the sperm membrane contains a high level of polyunsaturated fatty acids with susception of peroxidation. Peroxidative damage may result in loss of membrane functions and may lead to a reduction in fertilizing ability, motility and viability. Secondly, in contrast to other cells, spermatozoa have very limited ability to repair damaged structures, as a consequence of their small amount of cytoplasm and an active, highly condensed chromatin. Third, spermatozoa are equipped with a poor defence system against ROS; catalase is absent, and glutathione peroxidase and superoxide dismutase are present in relatively low amounts (Dohle, 2003).

To our best knowledge, only one retrospective cross-sectional study showing that genital tract infection may be a risk factor for semen quality in aging males has been published (Rolf *et al*., 2002). Infections of accessory glands were determined by positive bacteriological aerobic and⁄or anaerobic culture, screening for immunoglobulin A antibodies against chlamydia in seminal plasma, indicating an acute chlamydia infection, or leukocytospermia (>1.0 x $10^6$ leukocytes/mL) in combination with at least two other indirect signs of infection of the accessory glands, such as abnormal viscosity, elevated pH, or hypospermia. Data of more than 3,500 infertile men demonstrated that accessory glands inflammation occured in 6.1% of men under 25 years and 13.6% of patients over 40 years. Total sperm counts and semen volume were significantly lower in infertile subjects with infected accessory glands compared to men with idiopathic infertility. As ejaculate volume declined significantly in subjects with infections, but testicular volume remained unchanged, the post-testicular origin of impaired sperm count and semen volume, most probably due

to partial occlusion of the efferent ducts, must be considered causative. In addition, the proportion of subjects with infection increased with advancing age, providing more evidence that infertility is acquired due to infection, at least in some patients (Rolf *et al*., 2002).

#### 3.2.1.2. Prostate cancer

Only one previous study has investigated possible relations between semen quality and prostate cancer. In that study, 26 older men with histologically confirmed, organ-confined prostate cancer did not have altered seminal parameters compared to remaining 27 age-matched men without evidence of prostate cancer on biopsy (Ng *et al*., 2004).

#### 3.2.1.3. Benign prostate hyperplasia

Although we have prior scientific evidence associating LUTS, prostate enlargement and male reproductive functions, including erectile and ejaculatory dysfunction (McVary, 2006; Hellstrom *et al*., 2009, Gacci *et al*., 2011), there are currently no data associating LUTS and/or BPH with male seminal tract function and associated reproductive indicators (to our best knowledge).

### 3.2.2. Other chronic genital tract diseases

Increasing evidence suggests that chronic inflammatory conditions of the male genital tract (testicles, epididymis, and seminal vesicles) may not only impair basic sperm parameters, but also sperm functions and, subsequently, outcomes of in vitro fertilisation/intracytoplasmic sperm injection treatments. However, data showing that these diseases have negative influences on sperm quality and male fertility in general are controversial (Jungwirth *et al*., 2013). Also, the relations between chronic testicular inflammation and male infertility are under-investigated (Schuppe *et al*., 2010).

There are several pathophysiological mechanisms that may impair sperm function, including reduced accessory gland function, obstruction of sperm transport, the presence of unsuitable microenvironment in male genital tract, and dysregulation of spermatogenesis (La Vignera *et al*., 2011, 2014). For example, ROS and cytokines, products of neutrophil infiltration during infection, have roles defending against exogenous microorganisms, but may also contribute to tissue damage (Meinhardt, Hedger, 2011), seminal plasma hyperviscosity (Aydemir *et al*., 2008), and/or sperm dysfunction (Lampiao, du Plessis, 2009; Kullisaar *et al*., 2013) in infertile males.

Inflammation of the male genitourinary tract is a possible risk factor for impaired semen quality, and may affect sperm parameters to various extents, depending on the location in male reproductive tract. Moreover, different infectious agents may alter male accessory gland functions via inflammation (Mahmoud *et al*., 1998) and secondary obstruction (Dohle, 2003). These obstructions are usually incomplete and cause poor sperm quality, including low semen volume. Also, obstruction may cause epididymal occlusion, as well as

enlargement and cystic lesions of seminal vesicles, epididymis and prostate (Dohle, 2003).

Inflammation of the epididymis (epididymitis) in sexually active men under 35 years is most often caused by *Chlamydia trachomatis* or *Neisseria gonorrhoeae*. Nonsexually transmitted epididymitis is associated with urinary tract infection and occurs more often in men aged > 35 years, who have recently undergone urinary tract instrumentation or surgery, and in subjects with anatomical abnormalities (Jungwirth *et al*., 2013). Age-dependent, post-inflammatory alterations of the epididymis may lead to (partial) obstruction (Harris *et al*., 2011).

Also, the arteriographic impairments in the epididymis are correlated with systemic arteriosclerosis and may be age-related (Regadera *et al*., 1985). Epididymal alterations of various origins may lead to disturbed mitochondrial function and sperm dysmaturation (Aitken *et al*., 2007), giving possible explanation how sperm motility might be affected in older male.

### 3.2.3. Tumours and traumas in pelvic area

Malignant diseases may impair gonadal functions through hormonal alterations and metabolic conditions. Decreased reproductive hormones in cancer patients could result from stress, downregulation of endocrine substances produced by some tumours, or as a consequence of a metabolic condition. For example, in leukemia, lymphoma, and central nervous system tumours, the hypothalamus and the pituitary gland function may be directly impaired by tumour cell invasion or radiation therapy. Malignancies may also produce gonadal dysfunction via vitamin, mineral and/or trace element deficiencies. Moreover, tumour-released cytokines may affect spermatozoa, resulting in sperm alterations (Dohle, 2010). The main prognostic determinants of posttreatment fertility are the localisation of neoplasm and pretreatment semen quality (Trottmann *et al*., 2007).

Main sources of cancer in male genital tract are prostate and testicle(s). Associations between reproductive function and prostate cancer have been discussed in chapter 3.2.1.

Testicular germ cell tumour (TGCT), the most common malignancy in white men aged 15 to 40 years, affects approximately 1% of sub-fertile men. Testicular cancer represents 1% to 1.5% of male neoplasms and 5% of urological tumours in general. The incidence in Western countries is approximately 3–10 new cases in each year per 100,000 males, and varies between ethnic groups and countries. In the last 30 years, almost all Western countries have reported an increased incidence of testicular cancer. Epidemiological risk factors for testicular tumour development include prior infertility, history of cryptorchidism or undescended testicles (testicular dysgenesis syndrome), Klinefelter's syndrome, familial history of testicular tumours, and the presence of a contralateral tumour or testicular intraepithelial neoplasia (Albers *et al*., 2013; Jungwirth *et al*., 2013). Men with TGCT have decreased semen quality, even

before cancer is diagnosed (Petersen *et al*., 1999). Moreover, surgical treatment of TGCT results in additional impairment of semen quality (Jungwirth *et al*., 2013).

Of all genito-urinary injuries, one-third to two-thirds involve the external genitalia. Genital traumas are more common between the ages of 15 and 40 years. The main causes include road traffic accidents, physical sports, violent crime, and war-fighting (Summerton *et al*., 2013).

Spinal cord injury, predominantly seen in young men, is commonly related to neurogenic reproductive dysfunctions, including abnormal semen characteristics, ejaculatory failure and impotence. The causes are multifactorial and mainly post-testicular, including elevated oxidative stress, ROS and leukocyte levels in ejaculate, impairments in prostate and seminal vesicles milieu, postinjury bladder management, and autonomic nervous dysfunction, but all of them need further elucidation. Semen quality of men with spinal cord injury could impair as early as two weeks after injury. The main abnormality is seen in sperm motility not in sperm count (Patki *et al*., 2008).

### 3.2.4. General diseases and age-related changes in lifestyle

General diseases may eventually manifest in age-related health issues, including reproductive function, and may have a significant impact on the independence, general well-being and morbidity of men.

The effects of geography, smoking, alcohol use, ethnicity, and body mass index (BMI) on semen volume or sperm parameters in aging men were investigated in a study by Hellstrom *et al.* (2006). According to this study, geographic regions affected only sperm motility. There were no differences in semen parameters between smokers and nonsmokers. Similarly, moderate alcohol consumption did not affect semen parameters in aging subjects. However, according to the study by Eskenazi *et al.* (2003), semen volume was higher in men who never used tobacco or who never consumed alcohol. Similarly, smoking has been associated with increased ROS in the male genital tract (Sharma, Agarwal, 1996) and oligozoospermia (Harris *et al*., 2011).

The prevalences of obesity and metabolic syndrome (METS), the ever-growing and leading health problems worldwide, are significantly higher in men with in- or subfertility compared to normal population (Kasturi *et al.,* 2008). METS may decrease reproductive potential (Kasturi *et al*., 2008), including semen volume, sperm concentration and motility (Fejes *et al*., 2005[a]). Moreover, METS was shown to increase conversion of testosterone to estrogen through aromatase activity (Cohen, 2008), and may inhibit testosterone synthesis in testicles via chronic inflammation in the male genital tract (Kort *et al*., 2006). However, Hellstrom *et al.* (2006) found that BMI did not have significant effect on semen volume or other measured sperm parameters in men older than 45 years.

Some previous reports indicated that paternal occupation (farming, metal working and painting) may affect seminal function (Kenkel *et al*., 2001;

Magnusdottir *et al*., 2005). Also, anti-spermatogenic effects of ionizing radiation are well documented. Doses as small as 20 Gy depressed sperm output from proliferating spermatogonia, and doses above 75 Gy caused azoospermia (Nieschlag, Behre, 2010).

Possible links between mobile phones and semen parameters are detailed in few reports (Fejes et *al*., 2005[b]; Agarwal et *al*., 2008, Rago et *al*., 2013); however, the results in that topic are controversial and need additional research.

Alterations in semen quality can also occur after exposure to toxic agents (Wyrobek, 1993). For example, many older men have been exposed to dichlorodiphenyltrichloroethane and dibromochloropropane, endocrine disruptors that were used until 1970s (Kidd *et al*., 2001). Phthalates, nitroaromatic compounds and γ-diketones have been described as toxicants for seminal function (Nieschlag, Behre, 2010). Also, age-dependent increase of polychlorinated biphenyls was inversely correlated with sperm count and progressive motility in men with normal semen parameters (Dallinga *et al*., 2002). Among heavy metals, lead has been negatively associated with semen quality (Nieschlag, Behre, 2010).

Several cross-sectional studies have focused on the effects of human immunodeficiency virus (HIV) on sperm parameters (Dulioust *et al*., 2002; Diehl *et al*., 2003; Nicopoullos *et al*., 2004; Pavili *et al*., 2010). The results are controversial, but differences between HIV-infected and noninfected men, mainly in sperm motility and morphology, have been demonstrated. Moreover, changes in semen quality with disease progression have been debated (Dulioust *et al*., 2002; van Leeuwen *et al*., 2008; Rusz *et al*., 2012).

A study by Eskenazi *et al.* (2003) showed that sperm count and total number of progressively motile sperm were lower in men who had ever had high blood pressure. Some continuous medicines, including anti-hypertensives, $H_2$ blockers, anti-androgens, androgen replacement therapies, $\alpha_1$-blockers and 5-α-reductase inhibitors, may affect semen quality (Harris *et al*., 2011; Stewart, Kim, 2011). Marijuana, cocaine and opiates have minimal effect on spermatogenesis, but all narcotics could supress LH and testosterone secretion via inhibition of hypothalamic GnRH secretion (Nieschlag, Behre, 2010). Also, there is report that several chemotherapeutics have negative effect on spermatogenesis (Wallace *et al*., 2005).

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Hence, there are several unanswered questions concerning the factors that influene reproductive function in middle-aged men. The possible roles of couple infertility status, environmental and lifestyle-related factors are under-investigated. Moreover, associations between prostate conditions and male reproductive function are understudied and need additional studies.

# AIMS OF THE RESEARCH

The general aim of this work were was to specify reproductive function in middle-aged male and to find out possible influences of prostate conditions, lifestyle and couple's infertility status on male reproduction.

The specific aims of the research were:

1) To compare the reproductive parameters in middle-aged healthy men and male partners of infertile couples;
2) To discover the roles of lifestyle and education on reproductive function in middle-aged male;
3) To determine possible role of male genital tract inflammation on reproductive parameters in middle-aged male;
4) To reveal the impacts of lower urinary tract symptoms and prostate enlargement on semen quality and associated reproductive indicators in middle-aged male;
5) To evaluate reproductive function in male with premalignant and malignant prostate conditions;
6) To detect possible association between reproductive function and prostate-specific antigen in middle-aged male.

# MATERIALS AND METHODS

**Table 2.** Summary of study subjects.

| Study | Subjects | Investigations |
|---|---|---|
| Reproductive function in middle-aged healthy men versus male partners of infertile couples (Paper I) | 164 men with well-known status of couples infertility<br>61 men who considered themselves healthy and had fathered at least one biological child | Clinical investigation, basic semen analyses, blood samples for reproductive hormones, questionnaires about education, previous and current diseases, and lifestyle factors |
| Reproductive function and semen quality in middle-aged males: the role of non-malignant prostate conditions and genital tract inflammation (Paper II) | 347 men with LUTS, CBP and/or CP/CPPS<br>35 age-matched controls (without LUTS, CBP and/or CP/CPPS) | Clinical investigation, basic semen analyses, seminal interleukin-6 (IL-6) and microbiological investigation, blood samples for reproductive hormones and PSA, uroflowmetry, ultrasound for total prostate volume and residual urine, and symptom scores for prostate assessment |
| Decline of seminal parameters in middle-aged males is associated with lower urinary tract symptoms, prostate enlargement and bladder outlet obstruction (Paper III) | 380 men with LUTS<br>42 age-matched controls (without LUTS) | Clinical investigation, basic semen analyses, seminal IL-6, blood samples for reproductive hormones and PSA, uroflowmetry, ultrasound for total prostate volume and residual urine, and symptom scores for prostate assessment |
| Semen quality in middle-aged males: associations with prostate-specific antigen and age-related prostate conditions (Paper IV) | 122 men with increased serum PSA level<br>255 age-matched controls (with serum PSA level <2.5 ng/mL) | Clinical investigation, basic semen analyses, seminal IL-6, blood samples for reproductive hormones and PSA, uroflowmetry, ultrasound for total prostate volume, ans prostate biopsy for histological analyses |

# 4. Subjects and study design

Initially, we investigated the reproductive parameters, and lifestyle and educational indicators in male partners of infertile couples, attending the study between April 2000 and May 2010, and healthy men as the control group, included into study from November 2007 to October 2010.

Subsequently, we determined associations between male reproductive functions and prostate-related conditions in subjects who participated in screening for prostate health from November 2007 to February 2011.

The ages of all subjects in the study ranged from 45 to 69 years.

## 4.1. Male partners of infertile couples and healthy volunteers

### 4.1.1. Male partners of infertile couples

The study group included 198 males attending the clinic due to infertility of couples with no known female risk factors (Table 2). After the first data analysis, 21 men were excluded because their semen analysis or hormonal parameters were missing. Furthermore, subjects with a history of couple's infertility of less than 1 year (n = 5), with a prior varicocelectomy (n = 3) or with treatment for hernia inguinalis (n = 5) were excluded. Finally, according to World Health Organization definition (WHO, 2000), 164 subjects with couple's infertility (sexually active and without contraceptive use) lasting more than 12 months (mean ± SD 16.1 ± 2.0 months) were included in the study (Figure 5). Among them, 95 subjects were classified with primary infertility (no pregnancy at all), and 69 men with secondary infertility (unable to achieve pregnancy after the birth of at least one biological child) (WHO, 2000; Nieschlag, Behre, 2010).

### 4.1.2. Healthy volunteers

The control group consisted of men who considered themselves healthy and attended a prostate health screening at the Tartu University Hospital (Figure 5). In the initial phase, 364 men were in this group. Of those, 181 subjects were excluded according to the exclusion criteria (renal, hepatic, respiratory, endocrinological and/or cardiovascular diseases, or other prior chronic illnesses, continual use of medicine(s), prior or current problems and/or treatment for infertility or urogenital tumours, history of undescended testicle(s), previous varicocelectomy, hernio- or vasectomy, and chemo- or radiotherapy). Also, men that did not provide semen specimens (n = 122) were excluded. The number of subjects in the final group was 61. All males in this group were identified as fathers through self-administered questionnaire (completed within the screening period).

## 4.2. Subjects of prostate health study

In total, 639 men who underwent prostate health screening at the Tartu University Hospital were recruited for the study (Figure 5). Exclusion criteria for this study included prior or current problems and/or treatment of infertility or urogenital tumours, chemo- or radiation therapy in the pelvic region, previous varicocelectomy, hernio- or vasectomy, history of undescended testicle(s), and abnormal suspected findings from a digital rectal examination. None of the study subjects neither experienced febrile pelvic pain symptoms and acute urinary retention nor received therapy with antimicrobials, $\alpha_1$-blockers or 5α-reductase inhibitors within 3 months before the study.

### 4.2.1. Study of male genital tract inflammation

The final study group included only the males who were willing to provide semen specimens (n=411). Among them, 29 subjects with reported incomplete semen sample or insufficient hormonal or prostate-related clinical characteristics were excluded. The final study contained 382 men (mean age ± SD 55.9 ± 7.1 years).

To detect male genital tract inflammation, seminal white blood cells (WBC) counts and interleukin (IL)-6, an important marker of male accessory gland inflammatory process (Jungwirth *et al*., 2013) and the leading cytokine measured in seminal tract inflammation since 1990s (Comhaire *et al*., 1994), were determined.

Subjects were divided into three groups, based on their seminal WBC count. Two of them were separated according to cut-off level defined by receiver operating characteristics curve (ROCC) analysis (males with mild and moderate white blood cells count, i.e. WBC $<0.35 \times 10^6$/mL and $0.35–0.99 \times 10^6$/mL), presented in Paper II. The third group included subjects with leukocytospermic values that exceeded the WHO (2011) threshold for WBC count in semen ($\geq 1 \times 10^6$/mL).

Additionally, subjects were separated into two groups according to their seminal IL-6 levels. The cut-off level of IL-6 was calculated by ROCC analysis (IL-6 <57.0 ng/L and ≥57.0 ng/L), published in Paper II.

### 4.2.2. Study of male lower urinary tract symptoms and prostate enlargement

The final study group included males who were willing to provide semen specimens during initial (n=411) and repeated visits (n=37). Among them, 29 subjects with reported incomplete sample or insufficient hormonal or prostate-related clinical characteristics during their initial appointment were excluded, but three of them qualified after a secondary consultation and semen analysis. The final study contained 422 men (mean age ± SD 56.1 ± 6.7 years).

According to the International Prostate Symptom Score (I-PSS) questionnaire, 380 patients presented with LUTS (mean age 56.3 ± 7.2 years) and the remaining 42 subjects were without LUTS (mean age 55.8 ± 6.9 years). For further analyses, men were grouped according to I-PSS and total prostate volume (TPV).

Subjects were divided into four groups based to their I-PSS: men without LUTS (I-PSS 0) and with mild (I-PSS 1–7), moderate (I-PSS 8–19), and severe (I-PSS 20–35) symptoms (Oelke *et al*., 2013). Separation of subjects according to TPV was made using the cut-off levels (<30 and ≥30 mL), described in previous large-scale and long-term medical studies (Roehrborn *et al*., 2004; Roehrborn *et al*., 2011) and by risk for BPH progression (Emberton *et al*., 2008).

### 4.2.3. Study of histologically confirmed prostate conditions and prostate-specific antigen

The final study group included subjects who were willing to provide semen specimens (n=411). Among them, 29 subjects with a reported incomplete semen sample or insufficient hormonal or prostate-related clinical characteristics, and 5 males with diagnosis of prostate cancer with extracapsular extension (primary tumour stage ≥T3, Heidenreich *et al*., 2013) were excluded. The final study contained 377 men (mean age ± SD 55.9 ± 7.0 years).

To compare male seminal parameters with different serum PSA levels, subjects were divided into three groups according to risk for prostate pathologies (serum PSA level <1.0 ng/ml, 1.0–2.49 ng/mL, and ≥2.5 ng/mL) in middle-aged males (Loeb *et al*., 2006; Vickers *et al*., 2010; Lilja *et al*., 2011; Heidenreich *et al*., 2013). Among the subjects, 122 males presented with elevated serum PSA levels (Loeb *et al*., 2006; Heidenreich *et al*., 2013) and were separated into three subgroups according to prostate histological finding. The remaining 255 subjects with serum PSA level less than 2.5 ng/mL were defined as age-matched controls.

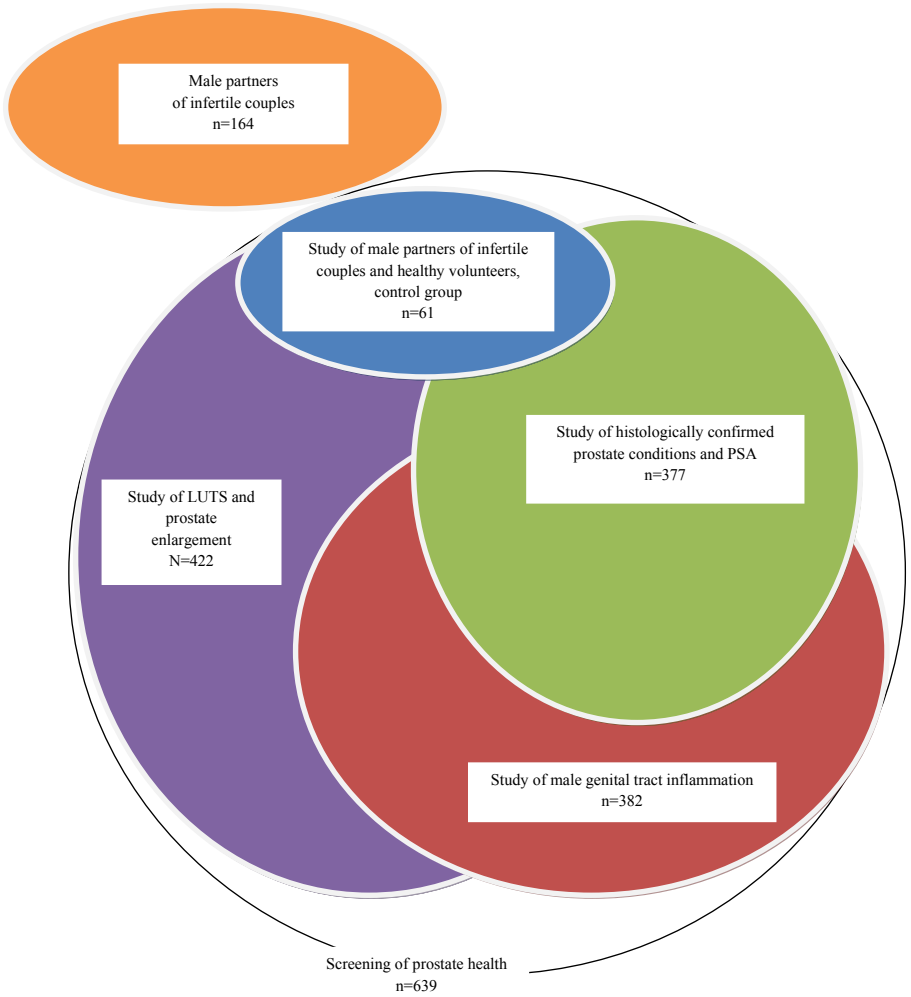


**Figure 5.** Subjects formation for the study of reproductive function in male partners of infertile couples and healthy volunteers, and for the study of reproductive function and prostate conditions.

# 5. Methods

## 5.1. Clinical examination

Physical examination included calculated body mass index (BMI) and genital pathologies (i.e. penis, testicles, groin and prostate, by digital rectal examination). BMI was calculated as weight in kilograms divided by height in meters squared. Mean testicular size was calculated as the mean volume of the right and left testicles, as measured with an orchidometer made of birch wood (Pharmacia & Upjohn, Denmark).

## 5.2. Questionnaires

Participants in the study completed questionnaires that included information about education, previous and current diseases, and lifestyle factors. In the prostate health study (chapter 4.2.), subjects completed symptom scores for prostate assessments, including the Estonian version of the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) score containing questions about chronic pelvic pain or discomfort, urination and bothersome symptoms (Korrovits *et al*., 2006), and the I-PSS (Oelke *et al*., 2013) for irritating, obstructive and, nocturnal LUTS.

## 5.3. Semen analyses

### 5.3.1. Basic semen parameters

Semen was obtained by masturbation and ejaculated into a sterile collection tube in a private room near the laboratory. Ejaculated semen was incubated at 37°C for 30 to 40 minutes for liquefaction.

All subjects were studied by the same criteria (WHO, 1999, 2011) at the Tartu University Hospital. Participants of the study were examined by one technician who had undergone extensive special training on laboratory standardization of semen sample analysis.

Routine semen analyses, performed according to WHO guidelines (WHO, 1999, 2011), included semen volume, total sperm count, concentration, motility, morphology, and WBC count. The recommended abstinence period before the ejaculation was a minimum of 48 hours but not longer than 7 days. The actual period of abstinence was calculated in full days between the current and previous ejaculation, as reported by the subjects.

Semen volume was determined by weighing the collection tube with the semen sample and subsequently subtracting the predetermined weight of the empty tube, assuming 1 g = 1 mL of semen. Motility assessment was performed in duplicate, and the average value of the two samples was calculated. Sperm concentration was assessed by the improved Neubauer haemocytometer. Morphology smears were stained with Papanicolaou method and spermatozoa were evaluated according to strict criteria (Menkveld *et al*., 1990).

### 5.3.2. Cytological analyses for detection of white blood cells

Semen smears for detecting WBCs were air-dried, stained with Bryan-Leishman method and examined by oil-immersion microscopy.

Seminal WBC concentration was calculated from known sperm concentration (as $10^6$/mL), according to the following formula:

(number of WBC counted/number of sperm counted) x semen sperm concentration

### 5.3.3. Detection of seminal interleukin-6

IL-6 levels in seminal plasma (100 μl of specimen was required for the assay) were measured with the Immulite automated chemiluminescence immunoassay IMMULITE 2000 analyzer (Kit Catalog Number L2K6P2, Immulite Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) according to the manufacturer's instructions. Solid-phase, enzyme-labeled sequential chemiluminescent immunometric assays were performed automatically on the IMMULITE 2000 analyzer, with two incubation cycles per 30 minutes, an analytic sensitivity of 2 pg/ml, and a calibration range of up to 1000 pg/ml. Granules coated with antibodies to IL-6 were mixed with the samples. Antibodies were highly specific to IL-6 and had no cross-reactivity with other interleukins, TNFα, or IFN-γ (Immulite 2000 IL-6). Intra- and inter-assay coefficients of variation were 4.7 and 5.3%, respectively.

## 5.4. Blood analyses

Venous blood was obtained from the cubital vein between 8 a.m. and 11 a.m. after an overnight fasting or light morning meal. Samples were centrifuged, serum was isolated, and specific markers were detected within 2 hours of collection at the United Laboratories of Tartu University Hospital.

### 5.4.1. Reproductive hormones

The levels of FSH (Kit Catalog Number L2KFS1), LH (Kit Catalog Number LKLH1), testosterone (Kit Catalog Number LKTW1), $E_2$ (Kit Catalog Number LKE21) and SHBG (Kit Catalog Number LKSH1) were measured in blood plasma with the Immulite Automated Chemiluminescence Immunoassay IMMULITE analyzer (Immulite Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA), according to the manufacturer's instructions.

Solid-phase, enzyme-labeled sequential chemiluminescent immunometric assays were performed automatically on the IMMULITE analyzer with one incubation cycle per 30 minutes for FSH, LH, and SHBG, and one incubation cycle per 60 minutes for testosterone and $E_2$. Calibration ranges were 0.1–170 IU/L for FSH, 0.1–200 IU/L for LH, 0.7–55.0 nmol/L for testosterone, 73.0–7,342 pmol/L for $E_2$, and 0.2–180 nmol/L for SHBG.

Intra- and inter-assay coefficients of variation were 4.2 and 8.0% for FSH, 4.0 and 7.1% for LH, 6.3 and 9.4% for testosterone, 7.5 and 13% for $E_2$, and 3.4 and 4.1% for SHBG (Immulite 2000 FSH, 2005; Immulite/Immulite 1000 LH, 2006; Immulite/Immulite 1000 Total Testosterone, 2006; Immulite/Immulite 1000 Estradiol, 2006; Immulite/Immulite 1000 SHBG, 2005).

#### 5.4.2. Prostate-specific antigen

PSA levels (Kit Catalog Number L2KUP6) in blood plasma were measured with the Immulite Automated Chemiluminescence Immunoassay IMMULITE analyzer (Immulite Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA), according to the manufacturer's instructions. Solid-phase, enzyme-labeled sequential chemiluminescent immunometric assays were performed automatically on the IMMULITE analyzer with two incubation cycles per 30 minutes. The calibration range was 0.003–20 ng/mL. Intra- and inter-assay coefficients for variation of PSA were 0.8 and 2.7%, respectively (Immulite 2000 Third Generation PSA, 2006).

### 5.5. Ultrasonography and uroflowmetry

All men in the prostate health study (chapter 4.2.) measured for main prostate parameters (Oelke *et al.,* 2013), including TPV and postvoided residual urine (PVR) by trans-rectal or abdominal ultrasound (using Logiq 5 Pro by General Electric, Milwaukee, WI, United States) and urinary flow rates by uroflowmetry (using Urodyn 1000 by Medtronic, Minneapolis, MIN, United States).

### 5.6. Histological analyses

In the prostate health study, subjects with serum PSA levels of at least 2.5 ng/mL (Loeb *et al*., 2006; Heidenreich *et al*., 2013) were recruited for prostate 12-core transrectal ultrasound-guided biopsies within 1 month after the initial analyses. Biopsies were taken from right and left lobe of the prostate using standard-score schemes (Figure 6) and18-gauge biopsy needles (Pro-Mag by Medical Device Technologies Inc., Gainesville, FL, USA). The samples were examined at the Tartu University Hospital by the experienced pathologist(s).

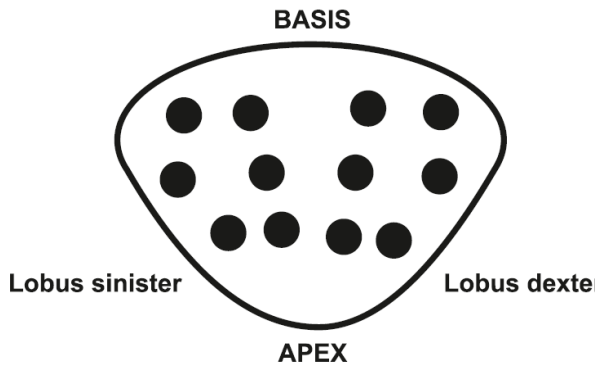


**Figure 6.** Algorithm of transrectal ultrasound-guided 12-core prostate biopsy.

### 5.7. Statistical analyses

For statistical analyses, SigmaStat (Systat Software, Chicago, IL, USA), Excel (Microsoft, Redmond, WA, USA) and R (R Foundation for Statistical Computing, Vienna, Austria) software programs were used.

Differences between groups were determined by the Mann-Whitney or Kruskal-Wallis tests. Group or groups that differed were isolated by multiple comparison procedures (Dunn's method). Spearman product moment correlation was used to determine correlations between age and reproductive or prostate-related parameters. According to WHO reference ranges for semen volume, sperm concentration, and sperm total motility (WHO, 2011), areas under ROCCs and diagnostic test characteristics (95% confidence intervals, CI) for seminal inflammatory markers were designed using R software to estimate semen pathology.

A multiple regression model was constructed to uncover significant effects of education, lifestyle factors, infertility/non-infertility status and age on reproductive parameters; and secondly, the relations between LUTS, male genital tract inflammatory markers and semen parameters were detected.

Education, lifestyle and environmental factors in male partners of infertile couples and healthy volunteers, using the Chi-Square or Fisher's exact test, were compared.

Statistical significance was assumed at $p < 0.05$ for all parameters.

### 5.8. Ethical considerations

Participation in the study was voluntary. Informed consent was obtained from all study subjects. The study was approved by the Ethics Review Committee on Human Research of the University of Tartu (certificate 166/T-14, 17.12.2007, No. 174/T-16, 22.09.2008 and 191/M-16, 29.03.2010).

# RESULTS AND DISCUSSION

## 6. Reproductive function in middle-aged male: association with lifestyle and couple's infertility

### 6.1. Semen parameters and related indicators in healthy men and male partners of infertile couples (Paper I)

In our study, serum $E_2$ levels, total sperm count and sperm concentration, proportion of morphologically normal sperms in semen, and testicular volume were significantly lower in male partners of infertile couples (n=164) compared to healthy men (n=61), the results are summarized in Table 3. Moreover, we found that subset of men with three normal variables (semen volume ≥1.5 mL, sperm concentration ≥15 x $10^6$/mL, and total motility ≥40%; WHO, 2011) was significantly lower among men in infertile couples compared to healthy subjects (41.2% versus 68.3%, $P < 0.001$). At the same time no significant differences in semen quality and related parameters between subjects with primary and secondary infertility were detected. To our knowledge, similar findings in males over 45 years have not been published previously.

Testicular size was in positive correlation with the main sperm characteristics in subjects of both groups. Although reduction of testicular volume is usually observed only after the seventh decade of life, some prior studies have described significantly lower testicular volume in chronically ill men compared to healthy subjects in a similar age group (Handelsman, Staraj, 1985). To our knowledge, there are no prior comparative data describing correlations between testicular size and semen parameters of men aged over 45 years.

Besides testicular volume, sperm concentration and morphology could be also good predictors of testicular function. Although previous studies (Ng *et al*., 2004; Auger *et al*., 1995) have demonstrated that decreased sperm count and a lower proportion of normal sperms are related to male aging, we did not find any significant influence of male age on semen quality and testicle volume in our study. Instead, we found differences in sperm parameters between healthy males and male partners of infertile couples. Therefore, it may be assumed that impaired sperm quality is not related to age in general, but to more specific physiological causes and lifestyle-related risk factors.

We also found that main sperm parameters were in negative correlation with serum FSH and LH levels in both groups. Correlation coefficients for male age, reproductive hormones, mean testicular size, and semen characteristics for both groups are presented in Table 2 of Paper I.

### 6.2. Impacts of general health, lifestyle, and educational factors on reproductive function (Paper I)

According to the data obtained from self-completed questionnaires on lifestyle risk factors, education, and general health, we determined that physical activity and sexual capability were higher in healthy men, whereas coital frequency and

history of sexually transmitted diseases were higher in men of infertile couples (Table 3 in Paper I); education, smoking and alcohol use were similar in both groups. Previous results from studies of older males are limited (Eskenazi *et al*., 2003; Hellstrom *et al*., 2006), and data from younger subjects are controversial. For example, the study of infertile couples from Künzle *et al.* (2003) showed that smokers had about 20% lower sperm concentration and total sperm count compared to non-smokers, whereas current smoking status had no independent effects on semen quality in young European males (Jensen *et al.,* 2004). Therefore, additional further and detailed investigations in this topic are needed.

We also found that sexual capability was in significant positive correlation with total sperm count ($r=0.368$, $p=<0.001$), sperm concentration ($r=0.386$, $p= <0.001$) and sperm motility ($r=0.194$, $p=0.020$), whereas physical activity was positively correlated with sperm concentration ($r=0.267$, $p=0.001$) and negatively correlated with semen volume ($r=-0.229$, $p=0.006$).

Multiple regression analysis was performed subsequently to uncover significant effects of education, lifestyle factors, status of infertility/non-infertility and age on reproductive parameters. Analysis confirmed positive associations of total sperm count and sperm concentration with sexual capability ($p=0.001$ and $p=0.006$, respectively), and a negative association between physical activity and semen volume ($p=0.033$). Also, intercourse frequency was negatively correlated with total sperm count and sperm concentration ($p=0.024$ and $p=0.033$, respectively). According to these findings, we assume that semen quality in men over 45 years may be related to certain lifestyle risk factors and needs additional detailed investigations.

# 7. Reproductive function in middle-aged males: association with prostate-related conditions

## 7.1. Role of genital tract inflammation (Paper II)

Subjects were grouped according to seminal WBC count and IL-6 levels (chapter 4.2.1.), these two markers were in positive correlation ($r=0.185$, $p<0.001$).

Semen volume, total sperm count and sperm motility were lower in patients with genital tract inflammation (Tables 5 and 6 in Paper II). Both seminal inflammatory markers (WBC count and IL-6 level) were in negative correlation with semen volume and total sperm count for all subjects. Moreover, WBC count was negatively correlated with sperm motility (Table 2 in Paper II).

Seminal IL-6 levels showed positive correlations with age and TPV, and both seminal inflammatory markers were positively correlated with serum PSA level (Table 2 in Paper II),. Although results of prior studies are controversial (Hochreiter, 2008; Loeb *et al.,* 2009), similar associations between semen parameters and seminal inflammatory markers were demonstrated in studies of younger, asymptomatic subjects (Korrovits *et al*., 2008), and between serum PSA levels and seminal inflammatory markers in expressed prostatic secretion of aging males (Ausmees *et al*., 2009[a]). Statistically significant correlations

between semen volume, sperm parameters and seminal inflammatory markers are presented in Figure 7.

Additionally, we determined seminal WBC counts and IL-6 levels in subjects with normal and declined semen parameters, defined by WHO reference ranges (2011). IL-6 levels in semen of men with three normal semen variables (semen volume ≥1.5 mL, sperm concentration ≥15 x $10^6$/mL, and total motility ≥40%) were significantly lower compared to subjects with at least one abnormal sperm parameter (mean ± SD 49.8 ng/mL ± 38.4 versus 90.4 ± 150.3 ng/mL, respectively, p=0.001). The seminal WBC counts were similar for both groups.

One of the striking findings in our study were higher serum $E_2$ levels and estradiol-to- testosterone ratios in patients with elevated seminal inflammatory markers (Tables 5 and 6 in Paper II). To our knowledge, no prior studies report hormonal levels in subjects with genital inflammation. However, there is evidence from animal models that prostatitis may change the balance of sexual hormones (Stoker *et al*., 1999; Cutolo *et al*., 2004).

### 7.2. Roles of lower urinary tract function and prostate enlargement (Paper III)

Subjects were grouped according to I-PSS and TPV (chapter 4.2.2.). We found that total sperm count and sperm concentration decreased as I-PSS and TPV increased; moreover, semen volume decreased in case of elevated I-PSS (Tables 1–1 and 1–2 in Paper III). The same tendencies were revealed with correlation analyses, where lower urinary tract symptom scores (I-PSS and NIH-CPSI) and TPV were negatively associated with semen parameters (Table 2 in Paper III). Also, maximum urinary flow rate (QMAX) was in positive correlation with semen volume and in negative correlation with time of abstinence before sperm analyses. Statistically significant correlations between semen parameters and prostate-related assessments are presented in Figure 7.

Additionally, we determined I-PSS and TPV in subjects with normal and declined semen parameters, defined by WHO reference ranges (2011). Total prostate volumes for men with three normal semen variables (semen volume ≥1.5 mL, sperm concentration ≥15 x $10^6$/mL, and total motility ≥40%) were significantly lower compared to subjects with at least one abnormal sperm parameter (mean ± SD 29.2 ± 10.3 versus 38.4 ± 14.1 mL, respectively, p=0.026). Similar differences were found for total I-PSS (mean ± SD 9.3 ± 5.8 versus 14.4 ± 7.3 score-points for subjects with normal and at least one abnormal sperm parameter, respectively, p=0.003).

Although urinary dysfunction is a classic clinical symptom related to prostate pathologies (Oelke *et al*., 2013), and associations between prostate and reproductive functions are presumable, the connections between LUTS and reproductive quality in middle-aged men have not yet been described.

### 7.3. Impacts of premalignant and malignant prostate conditions (Paper IV)

Subjects were grouped according to serum PSA levels and prostate histological findings (chapter 4.2.3.). We found significantly decreased sperm concentrations and total sperm counts in males with premalignant and malignant prostate conditions, compared to subjects without (pre)malignancies and age-matched controls. In addition, seminal IL-6 levels were increased in subjects with biopsy-confirmed BPH and/or chronic prostatitis, compared to age-matched controls (Table 2 in Paper IV).

Several controversial epidemiological studies about the relationships between male infertility and prostate cancer exist in the literature (Harlap *et al*., 2007; Jorgensen *et al*., 2008; Walsh *et al*., 2010; Ruhayel *et al*., 2010). Although the topic is relevant, there is only one clinical study that included 26 older men with histological diagnosis of organ-confined prostate cancer. The prostate cancer group had similar seminal parameters as the 27 subjects in age-matched control group with no evidence of prostate cancer (Ng *et al*., 2004). However, our study found that semen quality and related reproductive indicators were impaired in subjects with premalignant and malignant prostate conditions, compared to men without (pre)malignancies and age-matched controls. These alterations may be due to changes in general health status, malnutrition and gonadal function (Dohle, 2010), but may also be due to (partial) obstruction of prostate and accessory glands. Therefore that topic warrants more detailed investigations.

An unexpected finding of our study was the elevated serum FSH level in subjects with premalignant and malignant prostate conditions (Table 2 in Paper IV). Although the connection between serum FSH and spermatogenesis is well-accepted (Jarow *et al*., 2011; Jungwirth *et al*., 2013), little is known about the relationships between serum FSH and prostate. Besides direct connections have not been reported, initial findings indicate that FSH may be expressed by prostatic cells and upregulate aromatase activity in prostate (Dirnhofer *et al*., 1998).

### 7.4. Associations between reproductive function and prostate-specific antigen (Paper IV)

We investigated serum PSA levels in 377 subjects. Increased serum PSA levels were negatively correlated with semen volume, total sperm count, and sperm concentration. PSA levels were also associated with TPV, urinary flow rate and seminal IL-6 levels (Table 1 in Paper IV).

Also, we compared serum PSA level in subjects with normal and declined semen parameters, defined by WHO reference ranges (2011). PSA levels in men with three normal semen variables (semen volume ≥1.5 mL, sperm density ≥15 x $10^6$/mL, and total motility ≥40%) were significantly lower compared to subjects with at least one lowered sperm parameter (mean ± SD 1.5 ± 1.4 versus 2.5 ± 2.5 ng/mL, respectively, p=0.002). Statistically significant correlations of semen parameters and serum PSA levels in middle-aged male are presented in Figure 7.

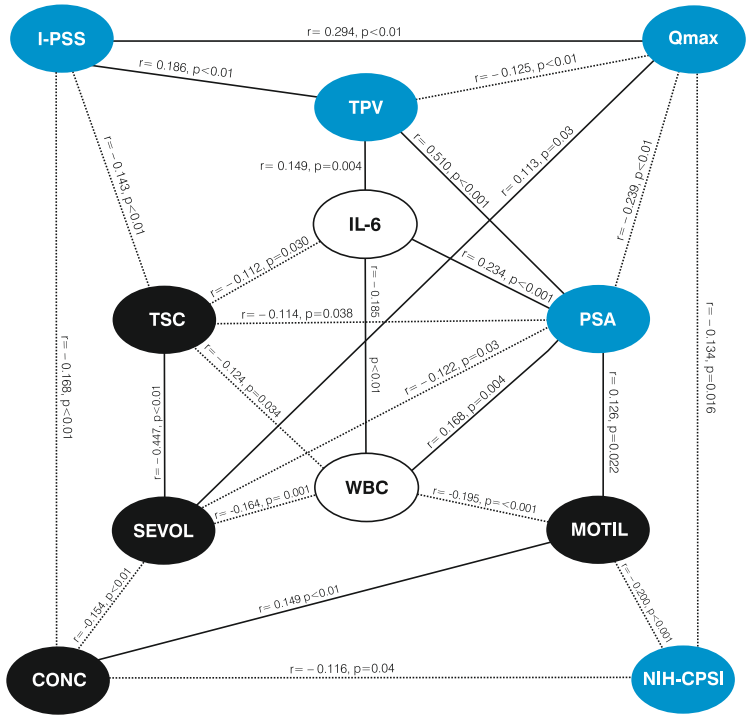


**Figure 7.** Statistically significant correlations between sperm parameters, seminal inflammatory markers and prostate-related assessments.

[1] Spearman rank-correlation coefficient

- sperm parameters (SEVOL, semen volume, TSC, total sperm count, CONC, sperm concentration, MOTIL, sperm motility)
- seminal inflammatory markers (WBC, white blood cells, IL-6, interleukin-6)
- prostate-related assessments (PSA, prostate-specific antigen, I-PSS, International Prostate Symptom Score, NIH-CPSI, National Institute of Health Chronic Prostatitis Symptom Index, QMAX, maximum urinary flow rate, TPV, total prostate volume)

We have not found prior reports associating serum PSA and seminal function in middle-aged males. Whereas similar data on younger subjects are also limited (Lackner *et al.,* 2006), this topic needs a more detailed investigation.

### 7.5. Multiple regression analysis of seminal and prostate-related parameters

Multiple regression analysis was performed in subjects who participated in the study of male genital tract inflammation, to uncover the significant effects of selected prostate-related parameters and seminal inflammatory markers on semen quality. Analysis indicated that sperm concentration, total sperm count and the proportion of normal spermatozoa were most of all influenced by prostate enlargement and total I-PSS, whereas sperm motility was associated with the total NIH-CPSI score and semen WBC count. The results are summarized in Table 4, indicating similar tendencies as revealed in above-described univariate analyses and in Tables 7 and 8 of Paper II, and Table 4 of Paper III.

**Table 3.** Age, testicular size, BMI, and basic semen and hormonal parameters (healthy men versus male partners of infertile couples).

| | Healthy men n=61 | Male partners of infertile couples n=164 | | | | |
|---|---|---|---|---|---|---|
| | A | B | B1 | B2 | A vs B | B1 vs B2 |
| | | Total n=164 | In couples with primary infertility n=95 | In couples with secondary infertility n=69 | *p* value[a] | *p* value[b] |
| Characteristics | Median (IQR) | Median (IQR) | Median (IQR) | Median (IQR) | | |
| Age (years) | 53.0 (49.0–56.3) | 50.0 (49.0–54.5) | 50.0 (49.0–53.0) | 50.0 (49.0–55.3) | 0.137 | 0.454 |
| Testicular volume[c] (mL) | 24.0 (22.0–25.0) | 22.0 (18.0–25.0) | 22.0 (18.0–25.0) | 22.0 (18.5–25.0) | 0.021 | 0.634 |
| BMI (kg/m$^2$) | 26.8 (24.7–29.5) | 27.1 (24.9–29.3) | 27.4 (25.0–29.9) | 26.0 (23.9–28.2) | 0.734 | 0.063 |
| **Basic semen parameters** | | | | | | |
| Semen volume (mL) | 2.8 (2.1–4.3) | 3.2 (2.0–4.5) | 3.0 (1.7–4.4) | 3.4 (2.3–4.7) | 0.768 | 0.396 |
| Total sperm count (x10$^6$) | 241.5 (150.7–452.3) | 97.8 (27.8–223.7) | 96.9 (30.0–248.4) | 98.8 (31.3–165.5) | <0.001 | 0.914 |
| Sperm concentration (10$^6$/mL) | 89.0 (47.8–179.3) | 29.5 (10.0–72.5) | 33.0 (9.5–85.3) | 26.0 (10.0–62.0) | <0.001 | 0.550 |
| Sperm total motility (%)[d] | 30.0 (18.8–32.3) | 28.5 (16.0–42.0) | 28.0 (13.8–41.3) | 30.0 (17.3–42.8) | 0.838 | 0.479 |
| Normal sperm (%) | 8.0 (2.8–9.3) | 4.0 (1.0–7.5) | 4.5 (1.0–10.0) | 4.0 (1.0–6.0) | 0.040 | 0.214 |
| WBC in semen (10$^6$/mL) | 0.0 (0.0–0.4) | 0.1 (0.0–0.2) | 0.1 (0.0–0.3) | 0.1 (0.0–0.2) | 0.768 | 0.621 |
| Abstinence time (days) | 5.0 (3.0–5.3) | 4.0 (3.0–5.0) | 4.0 (3.0–5.0) | 4.0 (3.0–5.0) | 0.207 | 0.197 |
| **Basic hormonal parameters** | | | | | | |
| Testosterone (nmol/L) | 16.4 (12.7–20.6) | 15.1 (11.2–19.8) | 16.2 (11.0–25.5) | 14.3 (11.5–18.9) | 0.207 | 0.430 |
| Estradiol (pmol/L) | 145.0 (113.8–180.5) | 94.3 (73.4–134.5) | 101.0 (73.4–138.8) | 90.5 (73.4–131.0) | <0.001 | 0.848 |
| FSH (IU/L) | 5.1 (3.4–7.3) | 5.3 (3.6–8.4) | 5.3 (3.8–8.3) | 5.2 (3.5–8.8) | 0.150 | 0.693 |
| LH (IU/L) | 2.6 (1.9–3.7) | 3.1 (2.2–4.6) | 3.0 (2.2–4.6) | 3.2 (2.1–4.6) | 0.998 | 0.667 |
| FSH/LH (IU/L) | 1.8 (1.3–2.6) | 1.9 (1.4–2.6) | 1.9 (1.4–2.5) | 2.0 (1.3–2.7) | 0.386 | 0.952 |

IQR, interquartile range (25th–75th percentile), BMI, body mass index, WBC, white blood cells, FSH, follicle stimulating hormone, LH, luteinizing hormone

[a] statistical difference between healthy men and male partners of infertile couples (Mann-Whitney test)

[b] statistical difference between male subjects in couples with primary and secondary infertility (Mann-Whitney test)

[c] right + left testicle/2

[d] according to WHO reference range (WHO, 2011)

**Table 4.** Relationship between prostate-related parameters, male genital tract inflammatory markers and semen parameters according to multiple regression analysis.

| | Semen parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Semen volume[a] (mL) | | Total sperm count[b] (million) | | Sperm concentration[c] (million/mL) | | Sperm total motility[c,d] (%) | | Normal sperm morphology[c] (%) | |
| Characteristics (n=382) | Beta | p value[e] | Beta | p value[e] | Beta | p value[e] | Beta | p value[e] | Beta | p value[e] |
| Total prostate volume (mL) | –0.004 | 0.622 | –2.451 | 0.048 | –0.542 | 0.102 | 0.005 | 0.940 | 0.008 | 0.711 |
| PSA (ng/mL) | –0.043 | 0.227 | –4.804 | 0.414 | –0.741 | 0.627 | 0.087 | 0.766 | –0.044 | 0.659 |
| Maximum urinary flow rate (mL/s) | 0.001 | 0.958 | 2.847 | 0.143 | 0.957 | 0.058 | 0.152 | 0.117 | 0.050 | 0.132 |
| I-PSS score (total) | –0.007 | 0.720 | –7.318 | 0.017 | –1.809 | 0.023 | –0.154 | 0.316 | –0.115 | 0.027 |
| NIH-CPSI score (total) | –0.009 | 0.562 | –0.217 | 0.936 | –0.460 | 0.511 | –0.303 | 0.025 | –0.004 | 0.937 |
| WBC in semen ($10^6$/mL) | –0.327 | 0.083 | –51.022 | 0.101 | –5.149 | 0.522 | –3.259 | 0.035 | –0.861 | 0.101 |
| IL-6 in seminal plasma (ng/mL) | –0.001 | 0.698 | –0.155 | 0.234 | –0.054 | 0.108 | –0.011 | 0.096 | –0.004 | 0.054 |

PSA, prostate-specific antigen, I-PSS, International Prostate Symptom Score, NIH-CPSI, National Institute for Health related Chronic Prostatitis Symptom Index, WBC, white blood cells, IL-6, Interleukin-6

[a] Adjusted R-square <0.01
[b] Adjusted R-square =0.04
[c] Adjusted R-square =0.03
[d] according to WHO reference range (WHO, 2011)
[e] p value gives the probability of this association

# GENERAL DISCUSSION

The main aim of our study was to define reproductive function and to identify possible influences of prostate conditions on male reproduction in middle-aged males. Initially, we compared reproductive function and educational, lifestyle-related and environmental factors of healthy subjects and male partners of infertile couples. Thereafter we examined the associations between reproductive function and different prostate conditions in middle-aged subjects who underwent the screening for prostate health.

The main finding in our study was that reproductive function in middle-aged subjects is not only related with general aging as described previously (Sartorius, Nieschlag, 2010; Stewart, Kim, 2011). The study indicated that semen parameters and associated reproductive indicators are associated significantly with certain lifestyle factors, including prior history of sexually transmitted diseases. In addition, fertile men had higher sexual capability and physical activity levels. This study also revealed significant differences in sperm quality and associated reproductive indicators, such as testicular size, between healthy men and male partners of infertile couples. Although reduction of testicular volume is generally observed only after the seventh decade of life, prior studies have described significantly lower testicular volume in chronically ill men compared to healthy subjects in similar age group (Handelsman, Staraj, 1985). In our study, similar tendencies were revealed when we compared males of infertile couples to healthy subjects. Moreover, the volume of testicles was positively correlated with the main sperm characteristics in both groups. To our knowledge, there are no prior analyses describing correlations between testicular size and semen parameters of men over 45 years old. Indeed, similar data on younger males are also limited.

Our study showed that serum $E_2$ levels were significantly higher in healthy men compared to males of infertile couples. To our best knowledge, similar findings for middle-aged males have not been published previously. However, it is known that estrogens may act via negative feedback on serum FSH and LH (Nicol *et al*., 2002; Pitteloud *et al*., 2008). Therefore, it is not surprising that one previous in vitro study has identified $E_2$ as a germ cell survival factor (Pentikainen *et al*., 2000). We also found altered serum $E_2$ levels in subjects with elevated seminal inflammatory markers in the study of prostate-related diseases and male reproductive function. While there are no available reports that specifically focus on hormone levels in male subjects with genital tract inflammation, evidence from animal models indicates that prostatitis may change the balance of sexual hormones. For example, Stoker *et al.* (1999) demonstrated the roles of perinatal oestrogenic exposure on prostate volume and increased prostatitis rates in rats. Otherwise, decreased testosterone level may also affect experimental prostatitis and suppress immune function in rat models (Cutolo *et al*., 2004). Also, our study of male genital tract inflammation and male reproductive function showed that increased seminal inflammatory marker (WBC count and IL-6) levels were associated with reduced semen parameters.

Although prostatitis and male genital accessory gland inflammation are not well-accepted causes of male infertility (Jungwirth *et al.*, 2013), there is strong evidence that male genital tract inflammation may affect reproductive function, including semen parameters (Shindel, Naughton, 2004; Engeler *et al.*, 2006), through (partial) occlusion of the seminal tract (Dohle, 2003).

According to our findings, reduced seminal parameters are also associated with prostate enlargement and LUTS in middle-aged males. These alterations may be related to changes in general health status, malnutrition and gonadal function (Dohle, 2010), but may also be due to subsequent (partial) obstruction of male accessory glands and/or prostate damage. Although the prostate, an accessory gland of the male reproductive system, is directly related to male reproductive, sexual, and ejaculatory functions (McVary, 2006; Hellstrom *et al*., 2009), there are only few clinical studies relating prostate-related conditions to reproductive function or sperm quality in middle-aged men (Rolf *et al*., 2002; Ng *et al.*, 2004; Ruhayel *et al.*, 2010; Walsh *et al.,* 2010).

In the literature and in clinical practice, relations between prostate pathologies and serum PSA levels are well-established (Sciarra *et al*., 2008; Oelke *et al*., 2013). Because we have initial results related with TPV, serum PSA level and seminal parameters (Ausmees *et al*., 2009[b]), we may assume that there should be connections between serum PSA levels and male reproductive parameters. In the present study, serum PSA levels correlated negatively with semen parameters, including semen volume, sperm motility, and total sperm count, and positively with seminal inflammatory markers, including IL-6 levels and WBC counts. Moreover, semen quality and related reproductive indicators were impaired in subjects with premalignant and malignant prostate conditions, compared to age-matched controls and subjects without (pre)malignancies. Therefore, according to these data, increased serum PSA levels in middle-aged males indicate not only prostate-related conditions, but may also desribe risks for impaired reproductive quality.

An unexpected finding of our study were elevated serum FSH levels in subjects with premalignant and malignant prostate conditions. Although there are reported connections between serum FSH and spermatogenesis (Jarow *et al*., 2011; Jungwirth *et al*., 2013), little is known about relations between serum FSH and prostate. To our knowledge, direct connections have not been reported. However, preliminary findings indicate that FSH may be expressed by prostatic cells to upregulate aromatase activity (Dirnhofer *et al*., 1998).

Interestingly, similar elevated serum FSH levels have been reported in subjects with *carcinoma in situ* of the testicles (Elzinga-Tinke *et al*., 2012). Despite analogous findings, we assume that elevated serum FSH is not (only) due to (pre)malignancy, but that complex aging process may affect via HPT axis, combining endocrine, cellular and structural alterations in male reproductive organs, including prostate and testicles.

The present study has some limitations. First, similar to most prior semen quality reports, our study included only men who attended a screening in outpatient clinic and were willing to provide semen specimens. Therefore,

included subjects may not accurately represent the general population of males over 45 years old. Secondly, because subjects of that age mostly do not wish to perform additional analyses, and participation in our study was voluntary, assessments of seminal parameters were performed on single semen sample (per appointment). Similar methods have been described in prior studies of men over 40 years old (Rolf *et al*., 2002; Ng *et al*., 2004).

Our study also has several strengths. Almost three-quarters of previous studies on reproductive function in middle-aged men did not consider the duration of abstinence before semen analyses (Sartorius, Nieschlag, 2010; Stewart, Kim, 2011). In our study, the recommended abstinence period (WHO, 1999, 2011) was 48 hours to 7 days for all participants. During the study, subjects were studied by the same criteria (WHO, 1999, 2011), in the same centre and laboratories. Also, age-matched controls were included in accordance with defined requirements (Engeler *et al*., 2006).

For further research, more detailed and prolonged studies are required to understand the causes of altered reproductive function in aging male, to promote ongoing healthy lifestyle, and to prevent life-long risk factors that may affect reproductive function.

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# CONCLUSIONS

Our study updated the current knowledge of reproductive function in middle-aged males and uncovered the role of prostate conditions in male reproduction. Some of these findings have not been described previously.

1) There are obvious differences in testicular volume, main sperm parameters and serum hormonal levels between men of infertile couples and healthy middle-aged males. Serum $E_2$ levels, total sperm count and sperm concentration, proportion of morphologically normal sperms in semen, and testicular volume are lower in male partners of infertile couples. These data revealed that sperm quality and associated reproductive parameters are not only related to general male aging as described previously.
2) Semen parameters in middle-aged males are associated with lifestyle-related influences, including physical activity and history of sexually transmitted diseases. Reproductive function of aging males may be improved by minimizing the life-long negative role of these risk factors.
3) Increased male genital tract inflammatory markers are associated with reduced semen parameters and increased serum PSA and $E_2$ levels. Similar results have been reported in younger men with asymptomatic prostatitis, indicating that decreased seminal function and elevated serum PSA may be due to prostate (partial) damage at younger age with long-term accumulation of inflammation-related influences thereafter.
4) Deterioration of seminal parameters in middle-aged men is associated with prostate enlargement and LUTS. Therefore, prostate volume is important not only for male urinary function and bothersome symptoms, but may also have important role in male fertility. Although assessments of prostate and LUTS may not replace semen analysis for evaluating male reproductive status, there is need for more detailed investigations to uncover the pathways behind these associations as well as possible related conditions.
5) Premalignant and malignant prostate conditions impair semen quality and related reproductive indicators. These alterations may be related to changes in general health status, malnutrition and gonadal function as described previously, but may also be associated with (partial) obstruction of prostate and accessory glands.
6) Elevated serum PSA levels are associated with decreased semen quality in middle-aged subjects. These findings, aAlthough results of prior epidemiological studies of male infertility and prostate cancer are controversial, these findings may suggest that infertile or subfertile young males should attend earlier prostate screening and baseline serum PSA measurement as commonly.

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# SUMMARY IN ESTONIAN

## Keskealiste meeste reproduktiivfunktsioon: seos eesnäärme, elustiili faktorite ja paari viljatusega

Mehe reproduktiivtervise uuringutele pööratakse kogu maailmas järjest rohkem tähelepanu järgnevatel põhjustel:

1) Sündivate laste vanemate keskmise vanus kasvab arenenud riikides erinevatel sotsiaal-majanduslikel põhjustel. Eelkõige mõjutavad seda hariduslikud ja tööalase karjääriga seotud otsused ning uue partnersuhtega seonduvad ootused lisanduvatele järeltulijatele;
2) Kehavälise viljastamise meetodite odavnemine ja kättesaadavuse paranemine. Eelkõige mõjutab see vanemas eas ning eelnevalt lastetute paaride reproduktiivsusega seotud otsuseid. Ligi 15% reproduktiivses eas paaridest on probleeme loomulikul teel rasestumisega, rohkem kui 1/4 neist juhtudest on seotud mehepoolsete põhjustega (Jungwirth *et al*., 2013).

Need muutused ning lisaks elanikkonna keskmise oodatava eluea tõusuga seonduvad meditsiinilised aspektid annavad meeste reproduktiivtervislikule käitumisele ja valdkonnaga seotud arengutele ka olulise sotsiaalse ning majandusliku sisu.

Kuidas vanus ja vananemine täpselt mehe reproduktiivfunktsiooni mõjutavad, on siiani vaieldav, sest puuduvad vastavad prospektiivsed uuringud. Varasemate kliiniliste uuringute alusel (Kidd *et al*., 2001; Kuhnert, Nieschlag, 2004; Sartorius, Nieschlag, 2010; Harris *et al*., 2011) võib öelda, et vanus on seotud seemnevedeliku mahu, spermide liikuvuse ja normaalsete seemnerakkude hulga langusega, kuid spermide kontsentratsiooni vanus oluliselt ei mõjuta (Kidd *et al*., 2001). Arvatakse, et vanusest sõltuvatel mehe viljakusnäitajate muutustel võib lisaks ealisele mõjule olla mitmeid lisanduvaid põhjusi – eelnevad traumad ja vigastused, vaskulaarsed, hormonaalsed ja metaboolsed haigused, organismi anatoomilised ja füsioloogilised muutused ning elustiili- ja keskkonnafaktorid.

Vanus on ka üks olulisemaid eesnäärmemahu suurenemise ja urineerimishäirete tekkega seotud riskifaktoreid. Ligi 50% viiekümne-aastastest meestest kurdab urineerimisega seotud kaebuste üle, alates viiekümnendatest eluaastatest tõuseb urineerimiskaebustega meeste osakaal ca 10% ühe eludekaadi kohta (Oelke *et al*., 2013). Teadaolevalt puuduvad hetkel uuringud eesnäärmemahu suurenemise, alumiste kuseteede kaebuste ning meeste reproduktiivfunktsiooni seoste kohta.

Eelnevad uuringud on viidanud, et mehe sugutrakti krooniline põletik mõjutab lisaks seemnerakkude kvaliteedi langusele ka paari viljakust ja kehavälise viljastamise protseduuride tulemusi. Siiski on tulemused esialgsed ja vastuolulised ning vajavad põhjalikumaid uuringuid (Jungwirth *et al*., 2013). Hetkel on meil andmed ainult ühe retrospektiivse uuringu kohta, mis kirjeldab sugutraktipõletiku rolli vanemate meeste viljakusnäitajate riskifaktorina. Rolfi (2002) poolt Münsteris viljatusprobleemidega meeste seas läbi viidud uuring

näitas, et sugutrakti põletike esinemissagedus alla 25-aastaste meeste seas oli 6% ja üle 40-aastaste meeste hulgas ligi 14%. Lisaks leiti nimetatud uuringus, et sugutrakti põletikuga meestel oli madalam spermide koguarv ja seemnevedeliku maht võrreldes meestega, kelle suguteedes põletikku ei leitud. Kuna võrreldud gruppides oli sarnane munandimaht, võib uuringu autorite arvates olla üheks vanemate meeste reproduktiivfunktsiooni languse põhjuseks kroonilisest põletikust tingitud suguteede (osaline) sulgus (Rolf *et al*., 2002).

Eesnäärmevähi ja mehe reproduktiivfunktsiooni võimalike seoste kohta on siiani avaldatud vaid üks uuring. Selles uuriti 52–79-aastaseid mehi, kellel oli eelneval histoloogilisel uuringul diagnoositud eesnäärmekasvaja (Ng *et al.*, 2004). Uuringus osalenud meeste viljakusnäitajad olid sarnased samas vanuses kontrollgrupi meeste viljakusnäitajatega. Samas on varasematest uuringutest teada, et kuse-sugutrakti kasvajalised muutused (nt munandivähk) mõjutavad oluliselt mehe reproduktiivsust ja järglaste arvu (Jungwirth *et al*., 2013) juba enne kasvaja diagnoosimist, mistõttu vajab eesnäärmevähi ja mehe reproduktiivfunktsiooni võimalike seoste ning ühiste riskifaktorite täpsustamine täiendavaid uuringuid. Teadmised keskealiste ja vanemate meeste reproduktiivfunktsioonist ning seda mõjutavatest faktoritest on kasinad, mis tingis vajaduse täiendavate uuringute järele.

## Uurimistöö eesmärgid

Uurimistöö eesmärgiks oli täpsustada keskealiste meeste reproduktiivfunktsiooni ning hinnata selle võimalikku seost erinevate eesnäärmehaiguste ja elustiilifaktoritega.

Uurimistöö täpsemad ülesanded olid:

1) Võrrelda keskealiste tervete meeste ja viljatute paaride meespartnerite viljakusnäitajaid;
2) Kirjeldada elustiili ja haridustaseme mõju keskealiste meeste viljakusnäitajatele;
3) Hinnata sugutraktipõletiku mõju keskealiste meeste viljakusnäitajatele;
4) Selgitada alumise kusetrakti sümptomaatika, eesnäärmemahu ning seotud mõjurite osa keskealiste meeste viljakusnäitajatele;
5) Määrata premaliigsete ja pahaloomuliste eesnäärmehaiguste mõju keskealiste meeste viljakusnäitajatele;
6) Täpsustada eesnäärme-spetsiifilise antigeeni võimalikku seost keskealiste meeste viljakusnäitajatega.

## Uuritav materjal ja meetodid

**Viljatute paaride meespartneritena** kaasati uuringusse 198 meest (vanus 45–60 eluaastat), kes osalesid kliinilises uuringus "Mehepoolse viljatuse põhjused". Uuringusse kaasamise kriteeriumiks oli varasem teadaolev paariviljatus rohkem kui ühe-aastase perioodi jooksul ning naisepoolsete riskifaktorite puudumine.

Peale esmast andmete analüüsi eemaldati uuringust 34 meest puudulike seemnevedeliku või vereanalüüside ning varem teostatud väikevaagna piirkonna operatsioonide tõttu. Lõplikku uuringugruppi kuulus 164 meest, kes täitsid terviseküsimustiku ning kellel teostati seemnevedeliku uuring ja vereanalüüsid suguhormoonidele. **Kontrollgruppi** kaasati uuritavad, kes osalesid uuringus "Eesnäärme haigused ja nende omavahelised seosed", hindasid küsimustiku alusel oma tervist heaks ja kinnitasid enda eelnevat teadaolevat bioloogilist isadust. Kokku vastas algkriteeriumidele 364 meest, kellest eksklusioonikriteeriumidele (teadaolevad eelnevad kroonilised ja kasvajalised haigused, operatsioonid väikevaagna piirkonnas, munandi laskumishäire ning keemia- ja kiiritusravi, püsiv ravimite tarvitamine ning probleemid paariviljatusega) alusel eemaldati 181 meest. Uuritavatest 122 meest keeldus andmast seemnevedeliku analüüsi, lõpliku kontrollgrupi moodustasid 61 meest. Kõik uuringus osalenud täitsid terviseküsimustiku ning neil teostati seemnevedeliku uuring (WHO, 1999, 2011) ja vereanalüüsid suguhormoonide määramiseks.

**Keskealiste meeste eesnäärmehaiguste ja reproduktiivfunktsiooni seoste uuringusse** kaasati algselt 639 meest, kes osalesid uuringus "Eesnäärme haigused ja nende omavahelised seosed". Uuringu eksklusioonikriteeriumideks olid eelnevalt teadaolevad kasvajalised haigused, operatsioonid ning keemia- ja kiiritusravi väikevaagna piirkonnas, munandi laskumishäire(d), probleemid paariviljatusega ning eesnäärmekasvajale viitavad muutused eesnäärme palpatsioonil. Uuringule eelneva 3 kuu jooksul ei esinenud kellelgi uuringus osalenutest uriiniretensiooni ning keegi ei tarvitanud antibiootikume, $\alpha_1$-blokaatoreid ja/või 5α-reduktaasi inhibiitoreid. Lõpliku uuringugrupi moodustasid mehed, kes olid nõus andma seemnevedeliku analüüsi. Viljakusnäitajate ja mehe sugutakti põletiku seoste uuringus osales 382 meest, viljakusnäitajate ja mehe alumise kusetrakti kaebuste ning eesnäärme mahu seoste uuringus 422 meest ning viljakusnäitajate ja PSA ning eesnäärme histoloogiliste muutuste seoste uuringus 377 meest. Uuringuskeemi kuulusid seemnevedeliku analüüs viljakusparameetrite ning põletikufooni hindamiseks (WHO, 1999, 2011), vereanalüüsid hormoonide ja organ-spetsiifiliste biomarkerite (PSA) määramiseks ning eesnäärme funktsionaalsust mõõtvad ultraheli ja kusevoolu kiiruse uuringud. Eesnäärme histoloogiline uuring teostati meestel, kelle seerumi PSA väärtus oli üle 2,5 ng/ml. Kõik uuritavad täitsid rahvusvahelised eesnäärmehaiguste küsimustikud (NIH-CSPI, I-PSS).

Uuring teostati SA Tartu Ülikooli Kliinikumi Androloogiakeskuses. Uuringus osalemine oli vabatahtlik. Uuring oli eelnevalt heaks kiidetud Tartu Ülikooli Inimuuringute Eetikakomitee poolt (luba 166/T-14, 17.12.2007, 174/T-16, 22.09.2008 ja 191/M-16, 29.03.2010).

## Uurimistöö tulemused ja järeldused

Meie uurimistöö täiendas seniseid keskealiste meeste reproduktiivfunktsiooniga seotud teadmisi ning täpsustas eesnäärme mõju mehe reproduktiivfunktsioonile.

1) Võrreldes tervete keskealiste meeste viljakusnäitajatega esinevad samaealiste viljatute paaride meespartneritel järgnevad olulised erinevused: naissuguhormoon östradiooli tase, spermatosoidide üldhulk ja kontsentratsioon ning morfoloogiliselt normaalsete spermatosoidide osakaal ja munandimaht on viljatute paaride meespartneritel oluliselt madalamad. Selle alusel võib järeldada, et viljakusnäitajate langus ei ole seotud ainult vanuselise komponendiga.
2) Elustiili küsimustike andmete alusel on regulaarne liikumisaktiivsus kõrgem tervetel keskealistel meestel, samas kui viljatute paaride meespartneritel on anamneesis rohkem läbipõetud seksuaalsel teel levivaid haigusi. Antud seosed tähendavad, et tervislikel eluviisidel on oluline mõju (vananevate) meeste viljakusnäitajatele.
3) Põletikufooni tõus mehe sugutraktis seondub seemnevedeliku koguse ning spermide koguarvu ja liikuvuse langusega ning tõstab PSA ja östradiooli taset seerumis. Kuna varem on sarnaseid seoseid leitud noortel asümptomaatilise suguteede põletikuga meestel, võib meie arvates üheks meeste viljakusnäitajaid ja seerumi PSA taset mõjutavaks põhjuseks olla noorte meeste eesnäärme ja suguteede põletikest tingitud kahjustus ning põletikufooni hilisem mõju mehe viljakusnäitajatele ja PSA tasemele.
4) Eesnäärmemahu tõus ja sellega seotud alumise kusetrakti (urineerimis)-kaebused seonduvad keskealiste meeste viljakusnäitajate langusega. Seega ei ole eesnäärme kontroll vajalik mitte ainult urineerimishäirete kontrolli ja üldise elukvaliteedi seisukohast, vaid aitab säilitada ja kaudselt jälgida ka mehe reproduktiivfunktsiooni.
5) Premaliigsed ja pahaloomulised eesnäärmepatoloogiad mõjuvad negatiivselt mehe seemnevedeliku näitajaid ja tõstavad seerumi FSH taset. Muutused võivad lisaks varem kirjeldatud üldtervise, toitumise ja munandi funktsiooni mõjudele olla seotud ka eesnäärme kahjustuse ja mehe suguteede (osalise) sulgusega.
6) Seerumi PSA taseme tõus on seotud keskealiste meeste reproduktiivfunktsiooni muutustega. Kirjeldatud seos võib tähendada, et subfertiilsed ehk langenud viljakusnäitajatega mehed peaksid enda PSA taseme jälgimist alustama soovitatavast vanusepiirist varem ning pöörduma PSA tõusu korral täpsustavale eesnäärmehaiguste uuringule.

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# PUBLICATIONS

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– Mehe reproduktiivfunktsioon
– Mehe reproduktiivtrakti patoloogiate omavahelised seosed
– Eesnäärmehaiguste omavahelised seosed

10 teaduspublikatsiooni rahvusvahelistes ajakirjades ja 25 suulist või poster-ettekannet rahvusvahelistel teaduskonverentsidel. Kolme raamatu (kaas)autor.

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