DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
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MALE FERTILITY AND ITS RISK FACTORS IN ESTONIA

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PRESS

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To my teachers:

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Outi
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and to my Family.

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LIST OF ORIGINAL PUBLICATIONS

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II. Punab, M., Zilaitiene, B., Jorgensen, N., Horte, A., Matulevicius, V., Peetsalu, A., Skakkebaek, N.E. (2002) Regional differences in semen qualities in the Baltic region.
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ABBREVIATIONS AND TERMINOLOGY

IVF	in-vitro fertilization
ICSI	intracytoplasmatic sperm injection
FSH	follicle stimulating hormone
LH	luteinising hormone
WBC	white blood cells
ROS	reactive oxygen species
WHO	World Health Organisation
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CV	coefficient of variation

Infertility is the inability or reduced ability to achieve a pregnancy after 12 months of trying and of unprotected intercourse

Subfertility is infertility without an absolute barrier to reproduction that would cause sterility

Primary infertility is a term used to describe a couple (or person) that has never been able to conceive a pregnancy after a minimum of 1 year of attempting to do so through unprotected intercourse

Habitual abortions are consecutive (three or more) pregnancies that end in miscarriage of the fetus

I. INTRODUCTION

Infertility is a unique category of human functional disability, where there have always been involved two persons, the female and the male. Fertility disturbances of one partner may only become evident through the other partners problem, while optimal reproductive function in one partner may compensate for impaired function in the other. Human fertility is not a constant parameter but it is dependent on age, current general health status, lifestyles, stress level, etc.

During some recent decades interest in the study and treatment of the causes of male infertility decreased substantially due to the introduction of effective methods of laboratory treatment as in-vitro fertilization (IVF) and in-vitro fertilization – intracytoplasmatic sperm injection (IVF-ICSI). In 1978 the first IVF baby in the world was born. A major breakthrough in the management of severe male infertility occurred in 1992 with the birth of the first baby conceived with the assistance of IVF-ICSI.

As a result, the trend has been bypassing of the evaluation of the male partner of an infertile couple and proceeding directly to IVF and/or ICSI. In majority of clinics worldwide the diagnosis of male infertility relies solely upon the findings of semen analysis. The results of semen analysis are usually interpreted by laboratory scientists (usually biologists) or gynecologists who know little about the clinical history or the clinical findings of real men. Changes in semen analyses among infertile patients are usually non-specific and give little indication of the cause of a man's infertility. The consequence of this approach is that men with medical pathologies as the cause of their sub-optimum sperm quality are not detected and treated properly.

On the other hand recent studies (Carslen et al., 1992; Auger et al., 1995; Irvine et al., 1996) indicate that a significant decline in male fertility potential as measured by semen quality has occurred during some last decades. There have been detected substantial geographical differences in semen quality. In the Northern European region significant differences in semen quality exist between Danish and Finnish men (Jørgensen et al 2001). However, there are no scientific data about the current situation and secular trends in male reproductive function and semen quality in Estonia.

Only a few studies (WHO, 1987; Pierik et al., 2000) have been designed to analyze the causes of male infertility and published so far. All publications have significant methodological shortcomings as lack of standardization of clinical and laboratory examinations, different referral levels of participating centers, etc.

The main purposes of the current thesis were to evaluate the reproductive function of young Estonian men and to detect the most important diseases and pathologies affecting the reproductive function of Estonian men.

Within the study 23 male subjects in Denmark, 965 young unselected men and 1537 infertile men in Estonia were investigated. The study was supported by the Estonian Science Foundation, the European Union, the Organon Agencies B.V. and the Danish Research Council and conducted in close collaboration with foreign colleagues from the University Department of Growth and Reproduction in Copenhagen; Institute of Endocrinology of the Kaunas University of Medicine; Institute of Biomedicine, Departments of Anatomy, Physiology and Pediatrics of the University of Turku and the Department of Obstetrics and Gynecology in Oslo Rigshospitalet. In Estonia, selection and recruitment of subjects and patients, collection and analysis of clinical samples, analysis and publishing of data took place in collaboration with colleagues from the Department of Surgery of the University of Tartu, the United Laboratories of Tartu University Hospital and the Andrology Unit of Tartu University Hospital.

II. REVIEW OF THE LITERATURE

1. Couple infertility

Infertility is commonly defined as the failure of conception after at least 12 months of unprotected intercourse (Rowe et al., 1993). It is usually accepted that about 10% of a random Western population of couples suffer from infertility (Mosher, 1982) but more recent publications summarizing existing evidence (Iammarone et al., 2003) present even higher values ranging from 10 to 16%. It is probable that this figure rises in the near future, as an increasing number of couples delay having children till an age when the natural fertility of one or both partners has declined, and secondly, sperm counts in Western societies continue falling (Carslen et al. 1992).

Unfortunately, there are few national data on the prevalence of infertility. Although census type data indicate the proportion of women and men remaining childless, they are confounded by the unknown proportion of voluntary childlessness.

No data are available so far on the epidemiology of infertility in Estonia.

2. Male factor infertility

The WHO carried out a multicenter study in 33 centers worldwide involving 7273 infertile couples in 1982–85 (WHO, 1987) and found that in 20% of the cases the problem was predominantly in the male, in 39% the problem was predominantly in the female, in 26% abnormalities were found in both partners, and in the remaining 15% no clear-cut cause of infertility was identified.

Diagnostic advances are progressively reducing the proportion of couples classified as having idiopathic infertility (deKretser, 1997). A survey (Thonneau et al., 1991) carried out among 1686 couples having consulted a gynecologist in three French departments in 1988 and 1989 for an infertility problem detected that in 38% of the cases abnormalities were found in both partners, in 34% of the cases the problem was predominantly in the female and 20% of the cases the problem was predominantly in the male. In that study the origin of couple infertility was not found in 8% of the cases.

Although problems of reduced fertility are common among men, 60% of men visiting a doctor for infertility have let more than two years pass before consulting a doctor about the problem (Bruchert, 1991).

Almost all studies on the epidemiology of (male) infertility are based on the clinical material of secondary or tertiary referral centers. Therefore we can not directly extrapolate the results of these studies to general population.

3. Semen quality as a marker of male reproductive function

Although semen analysis is routinely used to evaluate the male partner in infertile couples, sperm measurements that discriminate between fertile and infertile men are not well defined. Most existing data have been derived primarily from infertile or subfertile populations, from semen donors, or from men undergoing vasectomy. Widely used thresholds for normal semen measurements have been published by the World Health Organization (WHO, 1992; WHO, 1999), but in reality available norms for sperm concentration, motility, and morphology fail to meet rigorous clinical, technical, and statistical standards (Guzick et al., 2001). In recognition of these limitations, the nomenclature in the most recent WHO manual (WHO, 1999) for semen evaluation was changed from "normal" to "reference" values.

Several recent prospective studies of semen quality and fertility concluded that the current WHO reference values should be reconsidered (Bonde et al., 1998; Zinaman et al., 2000; Guzick et al., 2001). First, Bonde et al (1998) conducted a study on first-pregnancy planners in Denmark. They set a sperm concentration of 40 mill/ml as a reasonable threshold between subfertile and fertile men, since any higher sperm concentration was not associated with increased likelihood of pregnancy. However, the 95% CI around this threshold was broad and included a concentration of 20 mill/ml – the upper limit of oligospermia suggested by WHO (1992, 1999). There was significant correlation between the increasing proportion of normal sperm cells and an increasing likelihood of pregnancy when sperm concentration was higher than 40 mill/ml. Sperm motility is generally thought to be another important predeterminant of male fertility, but in the above study the proportion of non-motile sperm had a weak effect on likelihood of pregnancy. Semen volume was not related to likelihood of pregnancy except at very low volumes.

Second, Zinaman et al (2000) in their study followed prospectively couples after they discontinued the use of contraception. They detected that a decline in pregnancy rate occurred when sperm concentration was below 30 mill/ml of semen and the percentage of morphologically normal sperm decreased below 8% according to strict criteria (Menkveld et al., 1990).

In a third study Guzick et al (2001) identified subfertile ranges as a sperm concentration below 13.5 mill/ml, less than 32 percent of sperms with motility, and less than 9 percent of sperms with normal morphologic features. Fertile ranges were a concentration of more than 48 mill/ml, higher than 63 percent motility, and higher than 12 percent normal morphologic features. The values between these ranges indicated indeterminate fertility. In that study fertility was defined as pregnancy within the previous two years rather than current

pregnancy as it was defined in the two previous studies (Bonde et al., 1998; Zinaman et al., 2000).

Among the above mentioned parameters, sperm concentration is so far most commonly used to assess male fertility potential since its assessment is well standardized, less prone to subjective errors and quality control is easy to manage. Therefore, in our studies we focused mostly on sperm concentration as the most powerful and reproducible laboratory marker of male fertility.

4. Testis volume as a marker of male reproductive function

As the seminiferous tubules account for a great majority of the testicular tissue, testicular size reflects, to a certain extent, sperm production and thereby fertility. A WHO multicenter study on 7273 infertile men (WHO, 1987) detected that testis volume showed a significant linear correlation with sperm concentration ($r = 0.25$, $p<0.001$).

Unfortunately, due to the shortage of clinical andrologists and inherent difficulties with standardization of clinical male genital examination, this valuable marker of male fertility potential seldom occupies a deserved place in andrological investigations.

In the current study all clinical examinations of Estonian men were performed by an experienced andrologist and testis volume was used as an additional marker of male fertility potential in cases where more exact methods (semen analysis) were not available or accepted by the subjects of the study.

5. FSH and Inhibin-B as markers of male reproductive function

Follicle stimulating hormone (FSH) is a glycoprotein hormone secreted by the pituitary gland that controls development, maturation and function of the gonads. In an adult male FSH mediates concurrently with testosterone development of spermatozoa in the seminiferous tubules. For decades FSH has been served as the most reliable hormonal marker for assessing man's reproductive status but the serum level of FSH is influenced by hypothalamic function, testicular factors as well as by steroid hormones.

Inhibin-B is a glycoprotein hormone produced by the Sertoly cells in the testes. Its production depends on the number of Sertoly cells but is additionally influenced by germ cell factors and gonadotropins. In men Inhibin-B is the major regulator of FSH release in the pituitary gland.

In population studies on reproductive health of men both, FSH and Inhibin-B were concurrently used for the first time by Jensen et al (1997). In their study a

significant negative correlation was detected between FSH and Inhibin-B. Both hormones demonstrated comparably good and statistically significant correlation ($r = –0.40$, $p<0.01$ for FSH and $r = 0.38$, $p<0,001$ for Inhibin-B) with sperm concentrations. A recent study of Kumarov et al (2006) confirmed previous findings but noted that correlations of Inhibin-B levels with sperm parameters and testis volume were more significant than the correlation of FSH with these parameters.

In our study the reproductive function of young men in Estonia and in the broader Baltic-Nordic area, reproductive hormones were used as additional markers of male reproductive health.

6. Geographical differences and secular trends in semen quality

With the increased awareness of male factors as a cause of infertility, studies on human sperm quality gained scientific interest in the middle of the last century. Nelson and Bunge (1974) were probably the first to raise the problem that "something has altered the fertile male population to depress semen analysis remarkably". Similar findings have subsequently been reported by some other groups in Europe (Bostofte et al., 1983; Benvold, 1989) and North America (Leto and Frensilli, 1981).

In 1992 Carslen et al. published a meta-analysis based on 61 publications between 1938 and 1990, indicating a general decrease in mean sperm concentration from 113 mill/ml in 1940 to 66 mill/ml in 1990. At first, this article was criticized owing to possible methodological and statistical problems (Bromwhich et al, 1994; Olsen et al., 1995; Fish and Goluboff, 1997). Later, reanalyzes confirmed the original reported results (Bahadur et al, 1996). Addition of more recent publications to the data set used by Carlsen et al (1992) pointed to the possibility of an even more significant decline of sperm count (Swan and Elkin, 1999).

In recent years, new studies from Europe (Ginsburg et al., 1994; Auger et al., 1995; Irvine et al., 1996; Mencini-Fabris et al, 1996; Van Waeleghem et al, 1996) confirmed a decline in sperm concentration. Among these studies, Irvine et al (1996) and Auger et al (1995) observed that sperm concentrations were lower in men with a more recent year of birth in addition to a decreasing trend over calendar time. However, there are also reports from Europe confirming no change in sperm concentration, e.g. in the Toulouse region in France (Bujan et al, 1996) and in Finland (Vierula et al., 1996).

Additionally, it has become evident that significant differences in sperm count may exist even within one and the same country like France (Federation

Francoise des CECOS et al., 1997), Canada (Younglai et al., 1998) and the United States (Fish et al., 1996).

Although the methods used for assessment of sperm concentration have remained relatively constant since the introduction of the haemocytometer for sperm counting in 1929 (Macomber and Sounders, 1929), several quality control programs have reported significant interlaboratory differences in assessment results (Neuwinger et al., 1990; Matson, 1995). Thus, the interpretation of the data of retrospective studies is limited due to difficulties to assess interlaboratory differences and due to confounding factors such as age, ejaculation abstinence period and season. Furthermore, many of the recently published studies may be hampered by selection of special study subjects like vasectomy candidates (Fish et al., 1996), infertility patients (Bostofte et al., 1983; Vierula et al., 1996; Berling et al., 1997), semen donors (Auger et al., 1995; Bujan et al., 1996), or volunteers enrolled through advertisement (Irvine et al., 1996; Paulsen et al, 1996).

In 1995, a Copenhagen andrology group led by Prof. N.E. Skakkebaek initiated coordinated studies on male reproductive function in Northern Europe. All initiated studies were based on one common protocol. Prior to the studies the laboratory protocols were harmonized and external quality control program for assessment of sperm concentration ran constantly throughout the study period. In all studies assessment of sperm morphology and reproductive hormones was centralized. Northern-European studies focused mainly on two subject groups: young men – military recruits and partners of pregnant women.

In 2001, Jorgensen et al reported significant differences in the semen quality of partners of pregnant women in four cities in Western Europe: Turku, Copenhagen, Paris and Edinburgh. The lowest sperm concentrations and total counts were recorded for Danish men, followed by French and Scottish men. Finnish men had the highest sperm counts.

In 2000, Andersen et al published a Danish study on military conscripts showing a surprisingly low median sperm count, 41 mill/ml. The Danish study was performed in the urban areas of Copenhagen and Aalborg. In 2002, Richthoff et al published a study from the area of Malmö, a city in Southern Sweden, 20 km from Copenhagen, in which they reported 23% higher mean sperm concentration (53.8 mill/ml) in Swedish military conscripts compared to their Danish counterparts. Unfortunately, Swedish colleagues only used a recruitment protocol common with other Nordic-Baltic studies but they did not participate in the common quality control scheme. Therefore, it is finally difficult to compare their results with those of other studies from the same region.

The present study is the first attempt to describe male reproductive parameters of unselected young men in Estonia and in the broader Baltic-Nordic area, which is conducted according to a common study protocol and under continuous quality control.

7. Diseases and conditions influencing male reproductive function

Male infertility may be the symptom of a wide range of disorders. Due to the relative nature of both male and female infertility, it is difficult to define the exact distribution of the causes of fertility, particularly of disturbed male fertility. The importance of the correct diagnosis of all potential causes of male fertility is emphasized by the findings that men with a normal sperm concentration (over 20 mill/ml) but with abnormal motility or morphology of unknown origin have a 40% higher probability of achieving spontaneous conception than men in whom sperm abnormalities are related to a demonstrable cause such as varicocele, accessory gland infection, or congenital causes (Comhaire and Mahmoud, 2006).

Male infertility can be classified according to the topographic principle or according to the nature of the cause. However, in many clinics the diagnosis and also the classification of male infertility still relies solely upon the findings of semen analysis. The WHO has proposed a scheme for the diagnostic classification of the male partner of an infertile couple (Rowe et al 1993). This scheme, incorporating the results of semen analysis, clinical examination and hormonal tests, serves as a basis for standardization and for comparative multicentre studies. Still, recent advances in our understanding of the causes of male infertility, particularly in the area of genetic problems, indicate that there is a clear need to review previously used classifications.

To date the most comprehensive study on the causes of male infertility was carried out by the WHO in 1982–85 (WHO, 1987) including 33 centers worldwide and 7273 infertile couples. The largest single male diagnostic category in this study was the men with seminal abnormalities of unknown cause constituting over 45% of all established diagnoses. Besides this the most frequent diagnostic categories were varicocele (22.6%), accessory gland infection (12.4%), immunological infertility (5.4%), congenital abnormalities incl. cryptorchidism (3.0%), systemic causes (2.6%) and sexual dysfunctions (2.3%).

The distribution of diagnosis in patients attending infertility workup depends largely on the level (primary or referral center) and specialization of clinics or on the basic specialization of leaders of clinics. There is an elegant example with data (Nieshlag, 2001) from the Institute of Reproductive Medicine of University of Münster where due to the specialization and the tertiary referral level of the center, endocrine causes of infertility appear to be clearly overrepresented. In a referral andrology center in Rotterdam (Pierik et al., 2000) the most frequent diagnostic category among infertile men was also seminal abnormalities of unknown cause, accounting for 44.3% of study subjects. The most frequent causal factors were varicocele (14.2%), immunological infertility

(11.0%), accessory gland infection (5.2%), cryptorchidism and other congenital abnormalities of the male reproductive system (9.0%), and sexual dysfunctions (4.6%).

There are several other factors having the potential to influence male fertility such as advanced age, environmental exposures, smoking, and drug and alcohol abuse. Considering all these factors, we can conclude that the topic is controversial and we still lack a definite answer regarding their role in determining male (in)fertility.

Lack of specialists in male reproductive function/andrologists as well as serious problems related to standardization of the workup of infertile men makes correct analysis of the causes of male infertility difficult. Well-known shortcomings and wide variations in the distribution of the diagnosis of the causes of male infertility indicate the urgent need for new population based surveys in the field.

In our study we succeeded to eliminate major sources of biases common for studies on the causes of male infertility – centers of data collection were the only clinics offering andrological service in the area, the subjects were examined by one andrologist, and laboratory analyses were performed and interpreted according to common protocol and criteria.

7.1. Varicocele

Varicocele is formed of dilated veins in the pampiniform plexus of the spermatic cord. In general, a prevalence proportion of 15–20% is assumed in the male population and in approximately 30–40% of men presenting with infertility (Jarow, 2001). Although varicocele is a common problem in adulthood, it is rarely detected in prepubertal boys with an incidence rate of 2% to 11% (Kubal et al., 2004). The prevalence of varicoceles in pubertal men is comparable to that in adult population and suggests that physiological changes associated with puberty, such as an increase in testicular mass and in testicular blood flow, may play some role in varicocele formation (Sawczuk et al., 1993).

For anatomical reasons varicoceles occur more commonly on the left side. Bilateral varicoceles are present less frequently. Right-sided-only variceles are rare.

Varicoceles are usually identified in the man in the standing position. A painless compressible mass will be found superior, behind or rarely surrounding the testicle with increased turgidity in the veins as abdominal pressure is increased.

Many studies have been published dealing with the role of varicocele in male infertility, but the issue is still controversial. It is generally accepted that varicocele must be associated with abnormal semen quality in order to be accepted as a cause of infertility (Rowe et al., 2000).

The concept that the presence of a varicocele has a detrimental effect on fertility was supported by the existence of a relatively higher frequency of varicocele among the men with an infertile range of semen quality (25.4%, n=3626) than among men with normal semen quality (11.7%, n=3468) in a WHO study (WHO, 1992). Another argument that varicocele adversely affects male fertility is its association with ipsilateral testicular damage as reflected by reduced testicular volume. Since the time of Celsius it has been noted that testicles associated with large varicoceles have a reduced volume, which has been objectively documented in many clinical studies (Jarow, 2001). At the same time, studies on treatment of varicocele often report conflicting results. There is reliable scientific evidence that treatment of men with varicocele and subnormal semen quality improve the results of semen analysis and spontaneous pregnancy rate (Dubin and Amlar, 1975; Madgar et al, 1995). On the other hand, prospective studies failed to detect any positive influence of treatment of varicocele compared to counseling (Niesclag et al, 1998).

The progressive nature of infertility as a result of varicoceles can be proved by a significantly higher incidence of varicocele in the case of secondary infertility as compared to primary infertility (Gorelick et al., 1993). Also studies in pubertal boys (Haans et al., 1991; Paduch and Niedzielski, 1997) indicate that varicoceles appear to have a deleterious effect on testicular volume as well as on sperm number and quality from the very beginning of development of the disease and progress in severity over time.

So far the mechanisms by which varicocele affects testis function and male fertility have not been sufficiently explained. Elevation of testicular temperature and venous reflux from the renal vein containing toxic substances seem to play an important role in varicocele-induced testicular dysfunction. An inadequate function of valves in the internal spermatic vein may cause increased hydrostatic pressure in the testicular venules (Shafik and Bedeir, 1980), exceeding the pressure in the arterial capillaries and thereby reducing testicular perfusion.

Besides infertility, varicocele has been associated with the following andrological problems: failure of ipsilatral testicular growth and development, reduced testosterone level in older men (Comhaire and Vermeulen, 1975) and symptoms of pain and discomfort in the scrotal region (Waidner et al., 2002).

So far no one of epidemiological studies on varicocele testis has employed one and the same examiner using the same method of clinical examination in both control and study groups over the same time period.

7.2. Leukocytospermia

The prevalence and clinical significance of leukocytes (white blood cells, WBC) in semen is currently a matter of controversy. It is generally accepted that it is an indicator of inflammation in the genital tract. Leukocytospermia occurs frequently (10–44%) in infertile patients (Arata de Bellabarba et al., 2000; Stanislavov, 1999; Wolff, 1995; Omu et al., 1999; Sharma et al., 2001) and is associated with parameters of poor semen quality (Wolff et al. 1990; Arata de Bellabarba et al., 2000; Fedder, 1996; Ludwig et al., 2001). However, some investigators have failed to prove this (Aitken et al. 1992; Tomlinson et al., 1993). The diagnosis of leukocytospermia is usually based on the WHO definition of 1 x 10^6 WBC per ml of semen (WHO, 1992; Sharma et al., 2001). Leukocytospermia may affect male reproductive function in different ways. Inflammation can cause subtotal obstruction of the male reproductive tract (Dohle, 2003), and quantitative and qualitative reduction of spermatogenesis (Hales et al, 1999). Yet, probably the most important effect on the male reproductive potential is mediated through the effect of excessive levels of oxidative stress in semen (Aitken, 1994). Leukocytes produce at least 1000 times more reactive oxygen species (ROS) than spermatozoa (de Lamirande, 1995). Because of the high content of polyunsaturated fatty acids in the plasma membranes, spermatozoa are highly susceptible to oxidative stress (Aitken 1989). Polyunsaturated fatty acids are known to be toxic to spermatozoa and impair male fertility (Aitken, 1988; Wolff, 1995; Sharma et al., 2001; Pasqualotto et al., 2000).

Thus there are numerous unsolved questions in the area of male reproductive function and dysfunction in general and particularly in Estonia. There is no information about the general reproductive function of Estonian men in the light of recent negative trends in male reproductive function in Western societies. The knowledge of the distribution of the causes of male reproductive dysfunctions is insufficient in general and is missing for Estonian men. Data about the influence of major genital diseases and other potential reproductive risk factors on different markers of male reproductive function (semen quality, reproductive hormones, testes volume) are scarce or inconsistent.

III. OBJECTIVES OF THE STUDY

1. To improve the methods of standardization of the clinical data collected in multicenter studies on male reproductive function.
2. To study the markers of male reproductive function of young men in Estonia: testis volume, semen quality and level of reproductive hormones.
3. To compare the status of the reproductive function of Estonian men with the relevant characteristics of men in other Baltic-Nordic countries.
4. To find out the prevalence of the genital diseases affecting reproductive function in Estonian men.
5. To assess the informativeness of grouping of study subjects according to the count of spermatozoa in finding out the risk factors of male infertility.

IV. MATERIALS AND METHODS

1. Study subjects and methods of recruitment

Table1: Study subjects.

Publi-cation	Study site	Subject group	Subjects subgroups	Group number	Number of subjects	Goals of the study
Paper 1	UC	Subjects for quality control study		Group 1	23	Standardization of andrological clinical examination
Paper 2	UT	Military conscripts	Born 1979–81	Group 2	104	Assessment of the reproductive function of young men in Estonia
Paper 3			Born before 1979	Group 3	79	
		Soldiers		Group 4	118	
Paper 4		Control group		Group 5	664	Finding out of the prevalence of genital diseases and their influence on testis volume
		Infertile men	Infertile men (1997–99)	Group 6a*	779	
Paper 5			Infertile men (1997–2002)	Group 6	1537	Finding out of the prevalence of genital diseases in the case of various grades of male infertility

UT – University of Tartu
UC – University of Copenhagen
* subgroup of infertile men (group 6)

1.1. Subjects of quality control study

In Denmark 23 men with a mean age of 24 years (range 19±38 years) were selected from among participants in studies of reproductive function at Copenhagen University Hospital. They had been previously examined by one of the investigators who had also performed an ultrasound scan of the testes. They

were selected on the basis of having different testis sizes and scrotal pathologies and served as group 1 in the present study.

1.2. Military conscripts

In Estonia we invited men to participate in our study when they appeared for compulsory medical examination irrespective of the fact whether they were declared fit for service. The additional criterion for eligibility was that the men and their mothers were born in Estonia. Distinction between men of Estonian and of non-Estonian ethnicity was not taken into account. The study participants came from the city of Tartu or from the surrounding area. The investigation of the men lasted from November 1997 to May 1999. All men who participated in the entire study received 200 EEK as compensation. Among the men in Estonia attending compulsory medical examinations 183 (participation rate 19%) were eligible and agreed to participate in the study.

In studies on male reproductive function this group was divided according to the year of birth. To obtain groups comparable between centers collaborating in the study, all Estonian men born in 1979–81 (n=104) were enrolled in group 2 and their data were analyzed together with the data from the Nordic countries (paper 2). The mean age of the participants in the first group was 18.8± 0.8 years.

The rest of the cohort (n=79) formed group 3 and their data were analyzed together with the data of the Lithuanian cohort of military conscripts (paper 3). The mean age of the subjects in the second group was 20.4±1.0 years.

The results of the study of the Estonian men were analyzed together with the results for the military conscripts from Turku, Finland (n = 324, participation rate 13%), Oslo, Norway (n = 240, participation rate 17%) and Copenhagen, Denmark (n = 300, participation rate 19%) in paper 2 and together with the results for the military recruits from Kaunas, Lithuania (n=196 men, participation rate 14%) in paper 3.

Data collected: questionnaire, clinical examination, blood tests and semen analysis.

1.3. Soldiers

Because of the relatively low participation rates among the conscripts (groups 2 and 3), another group (group 4) with the same range of age – soldiers were included. The soldiers served in ordinary land forces near Tartu, and no special requirements for their physical health were required except being declared fit at compulsory medical examination. Thus, the soldiers may have had a better general health condition than conscripts from the general population. The

inclusion criteria were the same as for the conscript group. Among the soldiers 118 men (participation rate 75%) agreed to participate. The mean age of the soldiers was 19.8± 1.5 years. The soldiers who participated in the study received 200 EEK as compensation.
Data collected: questionnaire, clinical examination, blood tests and semen analysis.

1.4. Control group

The control group consisted of a subgroup of military recruit's who refused participation in the whole study and of young men with the same age range from secondary schools of Tartu and Valga. These men were offered to pass a much less demanding protocol where only a clinical examination was carried out. We followed the above described inclusion and exclusion criteria. The final control group (group 5) consisted of 664 men (participation rate 82%). Mean age of the men in the control group was 18.3± 1.6 years. No compensation was paid to the participants of this group.
Data collected: age, weight, height, clinical examination, history of major genital diseases and operations.

1.5. Infertile men

This study group (group 6) included a total of 1537 men who had visited the andrologist in the Andrology Unit of Tartu University Hospital or in Tähe Private Clinic (Tartu) due to involuntary infertility lasting over 12 months in the period between 01.01.1997–30.09.2002. The mean age of the participants was 31.1± 6.1 years. Approximately 90% of the men from infertile families who delivered semen samples in these clinics visited andrologist and passed a clinical examination as well and were included in study group. Less than 10% of the men presenting to the andrologist due to infertility refused or failed to deliver semen samples.

As there were no precisely defined criteria and information about the female partner was often missing or inconsistent, no attempts were made to distinguish between male and female infertility.

Considering the present knowledge of the influence of count of spermatozoa on male fertility (Bonde et al, 1998; Guzick et al., 2001), we divided the whole group into subgroups as follows:

1. azoospemia – no spermatozoa in seminal fluid (n=109)
2. cryptozoospermia – up to 0.1 million spermatozoa per ml of semen (n=20)

3. severe oligozoospermia – 0.1–5 million spermatozoa per ml of semen (n=92)
4. oligozoospemia III – 5.1–10 million spermatozoa per ml of semen (n=77)
5. oligozoospermia II – 10.1–20 million spermatozoa per ml of semen (n=174)
6. oligozoospermia I – 20.1–40 million spermatozoa per ml of semen (n=303)
7. normal sperm count (control group) – over 40 million spermatozoa per ml of semen (n=617)
8. subjects who did not deliver semen samples (n=145)

In order to minimize the effect of potential time dependent trend of differences on clinical examination, only the subgroup of men (n=779) from the time period accordant with the study of military recruits (1997–1999) was included in the study on the prevalence of genital diseases and their influence on testis volume (group 6a).
Data collected: health anamnesis, clinical examination, semen analysis, blood tests.

Participation in the study was voluntary. Ethical approval for the study was obtained from the Ethics Review Committee on Human Research of the University of Tartu, no. 53/31, 1997. All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

2. Methods

2.1. Clinical examination

All physical examinations were performed in accordance with a common protocol (Anderson et al, 2000) with the men in standing position. If needed, pathological findings were further clarified with the men in supine position. For assessment of testicular size, the orchidometer (made of birch wood, Pharmacia & Upjohn, Denmark) was used. Physical development was staged by the Tanner classification (Tanner, 1966). The position of the testicles in the scrotum, pathologies of the genital ducts (epididymis and ductus deference) and the penis, presence and grade of varicocele were registered for each study participant. Varicocele was graded according to a traditional system (Dubin and Amelar, 1971) as follows:

Grade I – palpated only on the Valsalva maneuver
Grade II– venous distension easily palpable but not visible
Grade III– venous plexus bulges through the scrotal skin, visible and palpable.

Assessment of cryptorchidism and operation due to inguinal hernia based on objective findings and interviewing. The subject's height and weight were recorded by an assisting nurse.

In Estonia all participants of the study were examined by one investigator (Margus Punab).

2.2. Semen analysis

2.2.1. Military recruits

Semen samples were assessed according to the criteria given by the WHO (WHO, 1992) which were slightly modified after Andersen and coworkers (Andersen et al., 2000). One technician performed all semen analyses. In a pilot study it became clear that as some men had long abstinence periods participation rate would decrease if we asked for an upper limit. Thus, the participants were asked to abstain from ejaculation for at least 48 h but were not given any upper limit. Semen samples were obtained by masturbation and were ejaculated into a clean collection tube, in a private room near the laboratories. After ejaculation semen was incubated at 37 C for 30–60 min for liquefaction. Ejaculate volume was estimated by weighing the collection tube with the semen sample and subsequently by subtracting the predetermined weight of the empty tube assuming 1 g = 1 mL. Motility was assessed in order to report the number of motile spermatozoa (WHO motility classes A + B + C). Sperm concentration was assessed using improved Neubauer haemocytometers and finally smears for morphology assessment were prepared. Following fixation and Papanicoulaou staining, the smears were sent to the Institute of Biomedicine, Departments of Anatomy, Physiology and Pediatrics of the University of Turku, Finland, for centralized morphology assessment according to strict criteria (Menkveld et al., 1990).

2.2.2. Infertile men

The semen samples of the infertile men were assessed according to the same criteria (WHO, 1992; Andersen et al., 2000) as those of military recruits, with only a few exceptions. The recommended abstinence period was 3–4 days. Generally, the first semen sample was used for analysis. When the first sample did not meet the standard criteria, e.g. the interval from the last ejaculation was less than 2 days or more than 10 days; part of the material was lost, or ejaculation was incomplete or unusual, the next appropriate sample was used for analysis when available.

Semen analyses were performed by three laboratory technicians, while the counting chamber of the same type (improved Neubauer) was used in all of these laboratories. Among basic semen parameters, we used in the current study

only sperm count as the most sensitive and the least subjective marker of male fertility, which was under strict and continuous external quality control throughout the whole study period.

For detection of leukocytes in semen, cytology smears were Bryan-Leishman stained and examined by an experienced microscopist using oil immersion microscopy (magnification = 1000x). The WBC concentration in semen was calculated using known sperm concentration (i.e. mill/ml) according to the following formula:

$$[WBC] = \frac{\text{number of WBC counted}}{\text{number of sperms counted}} \times \text{semen sperm concentration}$$

The test for estimation of leukocytes in semen was available only for 56.9% of the subjects who delivered semen samples.

2.3. Hormonal tests

2.3.1. Military recruits

Blood was drawn from the cubital vein. Serum was extracted and stored at –20° C until it was sent frozen to the Department of Growth and Reproduction in Copenhagen, Denmark, for analysis. The levels of follicle stimulating hormone (FSH) were determined by a time-resolved immunofluorometric assay (Delfia, Wallac, Turku, Finland). Testosterone was determined by a time-resolved fluoroimmuno assay (Delfia, Wallac, Turku, Finland) and Inhibin-B by a specific two-sided enzyme immunometric assay (Serotec, England). Intra- and interassay coefficients of variation for FSH were 3 and 4.5%, respectively. Both coefficients of variation were below 8% for testosterone. The intra- and interassay coefficients of variation were 15 and 18% for Inhibin-B.

2.3.2. Infertile men

Venous blood was obtained from the cubital vein. Sample was centrifuged, serum isolated and tests for reproductive hormones processed within 4 hours. FSH, LH, and testosterone and oestadiol levels of blood plasma were measured using the Immulite automated chemiluminescence immunoassay analyzer (Immulite DPC, Los Angeles, CA, USA) according to manufacturer's instructions. Among hormone tests in the current study only testosterone level was used to detect hypogonadism as the cause of male infertility. The intra- and interassay coefficients of variation (CV) were 16.3% and 24.3%, respectively for testosterone. The diagnosis of hypogonadism was established, if testosterone level was twice below normal limit (8 nmol/l).

2.4. Questionnaires (military recruits only)

Prior to the study, a standardized questionnaire had been developed in English (Andersen et al., 2000) and translated into Estonian. These translated questionnaires were back translated to check for translation errors. The questionnaires included information on age, previous or current diseases, including any known history of fertility, and some lifestyle factors

2.5. Genetic tests (infertile men only)

Cytogenetic analysis, tests for Y chromosome microdeletions and mutations in the CF gene were performed according to clinical indications in the United Laboratories of Tartu University Hospital and in the Institute of Molecular and Cell Biology of the University of Tartu. In cytogenetic studies, chromosomes of cultured peripheral blood lymphocytes were analyzed. Y chromosome microdeletions were analyzed according to the EAA guidelines (Simoni et al 1999). Eight most common mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene were analyzed.

2.6. Quality control

2.6.1. Semen analysis

Before the start of the study the technicians performing semen analysis in each center met in the University Department of Growth and Reproduction in Copenhagen to compare and to finally standardize the methods of analysis. All semen analyses of the study of military recruits in Estonia were performed by one technician.

Besides the common protocol and standardization meeting all centers of the study participated in an external quality control program coordinated by the University Department of Growth and Reproduction in Copenhagen throughout the study. Briefly, each month five blinded samples were dispatched from the Copenhagen laboratory. Fresh samples from the normal semen donors were preserved by addition of 10 μL of a 3mol/l sodium azide solution per 1 mL of the ejaculate after liquefaction, and finally 600 μL of semen was sent by mail in 1 mL cryotubes. This procedure was used in order to obtain undiluted samples, since the dilution step is considered an important source of variation in sperm counting. Usually, determination of sperm concentration was performed 4–8 days after semen preparation, including in the Danish laboratory. The results were reported to the Danish laboratory for statistical analysis.

Feedback about the results of the programme to the participating centers took place only after evaluation of the scientific results. The rationale for avoiding routine feedback was: information about the results of quality control might lead to changes in laboratory practice and if no feedback took place, then it was assumed that laboratories maintained their own determination level over time and the results from the quality control study could be used to correct the study results to achieve comparable levels between the laboratories.

These quality control results were used to adjust sperm concentration and total sperm count in order to present comparable levels.

2.6.2. Andrological clinical examination (Paper 1)

Unfortunately, only at the end of our multicenter study (on regional variation in male reproductive function) we realized that there are supraphysiological differences in the results of andrological clinical examination. To control and analyze these differences a special meeting was organized in Copenhagen. Altogether 23 men with a mean age of 24 years (range 19±38 years) were selected from among the participants in the studies on reproductive function at Copenhagen University Hospital. They had been previously examined by one of the Danish investigators. The subjects were selected on the basis of having different testis sizes and scrotal pathologies. On two successive days the subjects underwent an andrological examination by nine clinicians who performed the clinical investigations of the study subjects in their own centers.

The clinical examination followed the common protocol of the military conscripts study. The examinations were performed in a blind manner so that no investigator knew the results of the others. All data were collected for each day and the investigators were not allowed to see the results prior to the next examination.

3. Statistical methods

In the fertility studies of young men the unadjusted mean and median values, SD and 5–95th percentiles were calculated for the semen and hormone variables. The ejaculation abstinence period, men's age, year of birth, year of investigation and season of the year were evaluated as possible confounders for the semen parameters. For the percentages of sperm motility the time from ejaculation to assessment of motility was evaluated as an additional confounder. The hour of day of blood sampling was considered an additional confounder for the hormonal values. The effects of the possible confounders were tested by multiple regression analysis combined first for all three groups and thereafter for each of the groups separately. Sperm concentration and total sperm counts were normalized by natural logarithmic transformation before analysis to

correct for a skewed distribution. The percentages of motile spermatozoa were logit-transformed. All hormone parameters were natural logarithmic transformed to obtain a normal distribution of the results. Multivariate regression analyses were carried out to compare the groups. In these analyses the general level of each group was estimated for semen volume, sperm concentration and total sperm count while adjusting for duration of abstinence. The estimates of hormone levels were adjusted for the hour of day of blood sampling. The final models were subjected to standard check for residuals. The quality control data were normalized by natural logarithmic transformation followed by a two-way ANOVA. Between-group differences in self-reported information, regarding previous diseases, reproductive history, lifestyle factors, year of birth and year of investigation, season of investigation and time of blood sampling, were tested by the non-parametric chi-square test. Between-group differences in men's age, duration of abstinence and the time between ejaculation and assessment of motility were tested by the Kruskal–Wallis test.

In the studies on the prevalence of genital diseases and their influence on testis volume and on the causes of male infertility in Estonia statistical analyses were performed using SPSS version 10.1 for Windows. For comparison of the groups the χ^2 test was used. Statistical significance was assumed at $p< 0.05$ level for all parameters.

V. RESULTS

1. Quality control

1.1. Semen analysis

The results of the quality control study are presented in Table 2. The study revealed a significant difference between the five participating laboratories regarding assessment of sperm concentration ($P < 0.0005$). Any possible linear trend in laboratory estimate levels over time was also investigated and found to be non-significant. The results of analysis showed that compared to the Copenhagen reference laboratory the Estonian laboratory assessed sperm concentration 4% (95% confidence interval, −4; 13%) higher.

Table 2: Inter-laboratory differences (%) in assessment of sperm concentration as observed in the quality control study.

Countries	Sperm concentration	
	Difference (95% CI)	P-value
Estonia/Denmark	104% (96–113)	0.29 (NS)
Lithuania/ Denmark	115% (101–130)	<0.005
Finland/Denmark	111% (102–122)	0.02
Norway/Denmark	138% (126–150)	<0.005

Difference (95% CI): Relative difference and 95% confidence intervals (CI) in assessment of sperm concentration of quality control samples. For example, difference Estonia/Denmark of 104% shows that the centre in Estonia assessed the concentration 4% higher than the Danish centre.
NS = not significant.

1.2. Andrological clinical examination (Paper 1)

Figure 1 illustrates variation in measurement of testis size in three subjects with the testes with somewhat different size. It was found that the variation in measurement of testis volume increased with increasing testicular size. Analysis of variance revealed a statistically significant variation between the investigators (p=0.0001). Using these original datasets it can be estimated that the principal examiner from both Lithuania and Finland estimated testis size larger compared with the other examiners: 23 ml (median) versus 18, 19 and 20 ml for the Danish, Norwegian and Estonian principal examiners, respectively.

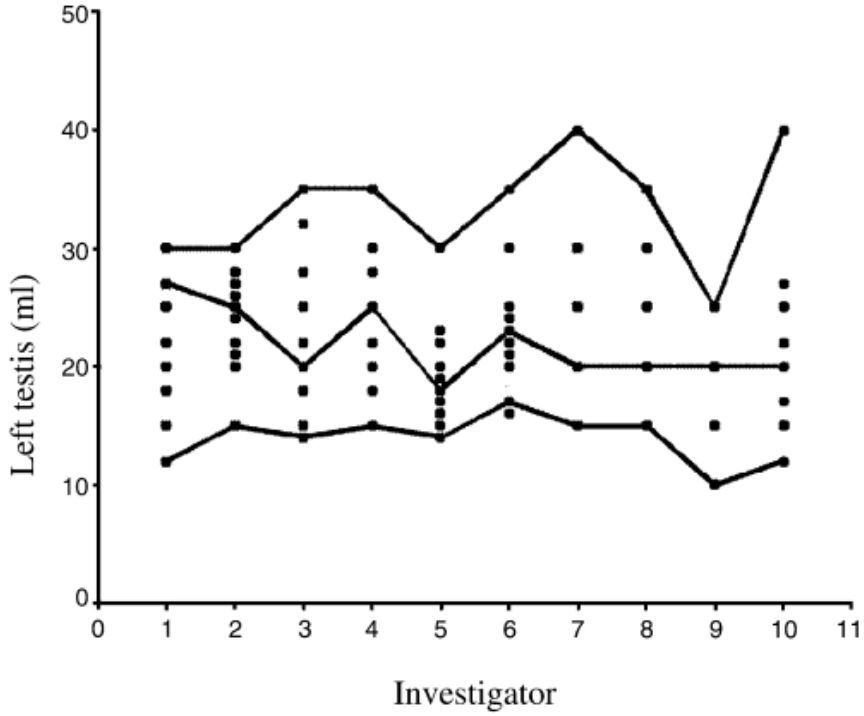

Figure 1. Individual measurements of left testicular size for all 23 men as measured by different investigators: 1–4 (Denmark), 5 (Estonia), 6 (Finland), 7–8 (Lithuania) and 9–10 (Norway). The drawn lines represent the measurements for three individual men to illustrate the measurement variation in for men with the testes of different size.

As can be seen in Table 3, the grading of the Tanner stages varied between the investigators from different countries. A left-side varicocele was found in three of the 23 men by six to eight of the investigators on the first day of examination. However, there was no clear consistency in the grading of the varicocele. Among the rest of the 23 men a grade 1–2 varicocele was diagnosed in 10 men by one to two different investigators.

Table 3. Tanner staging and number of men with scrotal abnormalities as indicated by the investigators from individual country. Only data from the first examination are shown

Tanner stages and Abnormalities	Denmark (1,2,3,4)	Estonia (5)	Finland (6)	Lithuania (7,8)	Norway (9,10)
Tanner stage					
4	0	2	0	0–3	0
5	0–4	10	1	3–4	1–3
6	19–23	11	22	17–19	20–23
Penis	0–3	1	0	0	0–1
Vas deferens					
Left	0–3	0	0	0	0
Right	0–2	0	0	0	1
Epididymis					
Left	1–4	4	1	0–2	1–3
Right	1–3	1	0	2	0
Hydrocele					
Left	1	1	1	0–1	1–2
Right	0–2	1	0	0	0
Varicocele					
Left grade I–III	3–5	4	4	3–4	1–5
Right grade I–III	0–1	0	0	0	0

2. Reproductive function of young men in Estonia and in the Nordic-Baltic area.

2.1. Estonian-Lithuanian study (Paper 2)

Semen parameters are summarized in Table 4. An increasing effect on semen volume, sperm concentration and total sperm count was seen during abstinence up to 96 h ($p = 0.005$, $p < 0.0005$ and $p < 0.0005$, respectively) after which no further increasing effect was seen for any of the groups ($p = 0.4$, $p = 0.8$ and $p = 0.5$). In addition to the observed values, Table 4 also presents adjusted values: for semen volume, sperm concentration and total sperm count, abstinence was taken into account as if the men would have had an ejaculation abstinence period of 96 h. Additionally, sperm concentration and total sperm count were adjusted – according to the results of quality control – to a level as if assessments had been undertaken in the Copenhagen laboratory. The Estonian men were assessed as having the higher semen volume, sperm concentration, total sperm count and percentage of normal sperms as compared to the Lithuanian men irrespective of the fact whether analysis was based on the entire study groups or on the subgroups without any known medical conditions.

Table 4. Semen parameters for the Estonian and Lithuanian men

	Observed		Adjusted			
	All men		All men		Subgroup	
	Mean (SD)	Median (5–95)	Median	95% CI	Median	95% CI
Semen volume (mL)						
Estonia, general population	3.3 (1.4)	3.0 (1.1–6.1)	3.3	2.9–3.9	3.5	3.0–4.1
Estonia, soldiers	3.6 (1.7)	3.1 (1.2–7.1)	3.3	2.9–3.7	3.3	2.9–3.7
Lithuania, general population	2.8 (1.6)	2.5 (0.5–6.0)	2.4	2.2–2.7	2.6	2.2–3.0
Sperm concentration (million/mL)						
Estonia, general population	81 (63)	64 (16–226)	67	54–82	66	52–84
Estonia, soldiers	106 (73)	88 (19–259)	82	69–97	78	64–94
Lithuania, general population	75 (55)	65 (12–192)	55	46–67	51	40–63
Total sperm count (million)						
Estonia, general population	269 (238)	218 (27–728)	223	172–290	232	171–314
Estonia, soldiers	400 (376)	269 (42–1237)	268	217–330	254	200–323
Lithuania, general population	208 (212)	145 (12–642)	133	107–165	130	99–171
Motile sperm (%)						
Estonia, general population	74 (10)	76 (56–87)	75	72–77	74	70–77
Estonia, soldiers	72 (13)	74 (48–88)	73	71–75	73	70–75
Lithuania, general population	76 (11)	79 (57–86)	77	75–78	75	72–77
Normal morphology (%)						
Estonia, general population	7.7 (5.3)	7.0 (1.0–19.2)	7.7	6.3–9.2	8.5	6.7–10.2
Estonia, soldiers	9.6 (8.6)	8.0 (1.0–23.8)	9.6	8.3–10.9	8.9	7.5–10.4
Lithuania, general population	6.2 (4.2)	6.0 (0.20–14.0)	6.2	5.1–7.3	5.5	3.8–7.2

Observed: the results are based on raw data; SD: standard deviation; 5–95: 5–95th percentile.
Adjusted: median and 95% CI (5–95% confidence interval) calculated for the individual groups by linear regression analysis. Sperm concentration and total sperm count are adjusted to the level of the Danish laboratory (according to the results of quality control) and adjusted to a period of ejaculation of at least 96 h. Morphology smears were all assessed in Finland by AH.
All men: the results are based on all men participating in the study.
Subgroup: the results are based entirely on men not taking any daily medication, men without any previous or current andrological diseases including known fertility problems. Information obtained from a self–administered questionnaire.

Table 5 summarizes the results of hormonal assessments. The Estonian men from general population had lower levels of Inhibin-B compared to the Lithuanian men and the Estonian soldiers had the lowest levels of Inhibin-B. The level of total testosterone in the Estonian and Lithuanian men from general population was almost the same but higher than in the Estonian soldiers.

Table 5. Hormone parameters for the Estonian and Lithuanian men

	Observed		Adjusted			
	All men		All men		Subgroup	
	Mean (SD)	Median (5–95)	Median	95% CI	Median	95% CI
FSH (IU/I)						
Estonia, general population	3.9 (2.1)	3.3 (1.6–8.4)	3.4	2.9–3.8	3.4	2.9–3.9
Estonia, soldiers	3.7 (1.8)	3.5 (1.3–7.3)	3.1	2.8–3.5	3.4	2.9–3.8
Lithuania, general population	3.6 (1.8)	3.2 (1.3–6.8)	3.0	2.7–3.3	3.2	2.7–3.6
Inhibin-B (pg/mL)						
Estonia, general population	220 (68)	221 (113–328)	218	200–239	219	197–243
Estonia, soldiers	185 (57)	184 (92–283)	186	172–201	184	167–203
Lithuania, general population	233 (92)	222 (117–388)	233	217–250	241	219–266
Testosterone (nmol/L)						
Estonia, general population	26 (8)	25 (13–38)	26	24–28	26	23–29
Estonia, soldiers	18 (6)	18 (7–29)	18	17–20	18	16–20
Lithuania, general population	26 (8)	25 (14–41)	27	25–29	27	25–30

All serum samples assessed in one laboratory.
Observed: the results are based on raw data; SD: standard deviation; 5–95: 5–95th percentile.
Adjusted: the estimates and 95% CI (95% confidence interval) calculated for individual groups by linear regression analysis. In assessment the hour of day of blood sampling is taken into account and adjusted to sampling carried out between 10 and 11 a.m.
All men: the results are based on all men participating in the study.

The possible confounding factors for the semen parameters differed significantly between the three investigated groups but only duration of abstinence had a statistically significant effect on semen volume, sperm concentration and total sperm count (all $p>0.0005$). Men's age, year of birth, year of investigation, season or 'time from ejaculation to assessment of motility' did not have a confounding effect and were therefore not included as confounders in final analysis. Hour of day of blood sampling had a statistically significant effect on the level of testosterone and Inhibin-B (all $p < 0.0005$) with the highest values in the morning hours and declining levels during the day. For the other hormones similar tendencies were seen, although statistically non-significant. Thus, hour of day of blood sampling was included as a confounder in the analysis of the hormone results.

Self-reported information obtained from the questionnaires is summarized in Table 6. Statistical comparisons between the men from 'general population' as well as comparisons between all three groups are presented. Only 7.8% of the Estonian men from general population and 0.9% (one person) of the Estonian soldiers had taken medication during the last 3 months before participation in the study. In contrast, 31% of the Lithuanian men reported to have taken some kind of medication prior to the study. Altogether 47.9% of the Lithuanian men were without any of the conditions mentioned in Table 4 in contrast to 70.9 and 70.4% of the two groups of Estonian men. These subgroups of men without any medical conditions comprise the 'subgroup' in Tables 4 and 5. Seventy per cent of the Estonian soldiers were smokers. Of the men from general populations, 55% of the Estonians and 56% of the Lithuanians were smokers. No effect of smoking was detected either on the semen parameters or on the hormones (data not shown).

Table 6. Self-reported conditions of young men from Lithuania and Estonia obtained from the self-administered questionnaire. The results are shown as frequencies (%)

	General population			Soldiers	p–value between all groups[a]
	Estonia (*n* = 79)	Lithuania (n = 196)	p–value	Estonia (*n* = 118)	
Been diagnosed as having[b]:					
Varicocele	7.1	0.5	0.002	1.9	0.006
Epididymitis	0.0	0.5	0.54	0.0	0.61
Gonorrhoea	0.0	1.1	0.05	2.8	0.26
Chlamydia	7.2	0.5	0.002	1.9	0.006
Orchitis due to parotitis	2.6	7.9	0.10	0.0	0.003
Cystitis	2.9	3.8	0.74	3.8	0.10
Diabetes	1.5	0.0	0.10	0.9	0.31
Thyroid Disease	1.6	1.6	0.99	0.0	0.54
Been operated or treated for[c]:					
Varicocele	1.4	0.6	0.48	0.0	0.47
Cryptorchidism	0.0	2.1	0.2	0.9	0.35
Other diseases of penis, urethra or scrotum	1.4	1.1	0.83	1.0	0.96
Inguinal hernia	1.3	3.8	0.3	1.8	0.42
Subject has:					
Had cryptorchidism, spontaneously descended[d]	2.4	1.1	0.5	0.0	0.49
Experienced fertility problems[e]	4.3	4.3	1.0	8.8	0.27
Caused a pregnancy[f]	8.0	13.0	0.3	16.7	0.22
Taken some medication during the last 3 months[g]	7.8	31.0	<0.0005	0.9	<0.0005
Subgroup of men not affected by any of above conditions[h]	70.9 (*n* = 56)	47.9 (*n* = 94)	0.001	70.4 (*n* = 83)	<0.0005

[a]Pearson chi-square test; [b]Questions were, e.g. phrased as 'Have you ever been diagnosed by a doctor as having varicocele?' No information about how the diagnoses were obtained; [c]Questions were, e.g. phrased as 'Have you ever been operated for varicocele?' No further specific information regarding treatment. For cryptorchidism operated or treated by hormones; [d]The question was 'Were you born with one or both of your testicles outside the scrotum, but they went descended spontaneously (without surgical or hormonal treatment)?'; [e]The question was 'Have you ever had regular unprotected intercourse for at least 1 year, without your partner becoming pregnant?'; [f]The question was 'Have you ever caused a pregnancy?'; [g]The question was 'Have you taken any medication (at any time) during the last 3 months (before participating in this study)?'; [h]Men responding negative to all categories of self-reported information.

The majority of all the investigated men had an adult pubic hair distribution (Tanner stage 5 or 6): 99% of the Estonians from general population, 89% Estonian soldiers and 98% Lithuanians. Median testis size (mean of left and right testis) was 23, 22 and 25 mL, and median body mass index was 22.0 and 21.8 and 21.9 respectively.

2.2. Nordic-Baltic study (Paper 3)

Semen parameters, based upon the raw data obtained in each city, are summarized in Table 7 as 'observed' values. In all four groups of men, increasing duration of abstinence had an increasing effect on semen volume, sperm concentration and total sperm count up to ~96 h ($P < 0.0005$ for all three parameters), whereafter no further effect of a longer abstinence period was observed. Neither the age of the man, his year of birth, year of investigation or season of investigation had any confounding effects on these parameters. Multivariate regression analysis accounting for the period of abstinence revealed no difference between the semen volumes of the men from the four countries, whereas sperm concentrations and total sperm counts differed significantly between the centers (all $P < 0.0005$) when results of the quality control were not taken into account. In the final calculations of sperm concentration and total sperm counts, corrections for these inter-laboratory differences were additionally included in the estimates (Table 7, 'adjusted' values). From these estimates it appeared that the men from Estonia (Tartu) and Finland (Turku) had a higher sperm concentration and total sperm count than the men from Norway (Oslo) and Denmark (Copenhagen). Statistically significant differences were shown for the values for Estonia versus Denmark (95% CI for the difference 1.07–1.79), for Estonia versus Norway (95% CI for differences 1.05–1.82), for Finland versus Denmark (95% CI for the difference 1.08–1.59) and for Finland versus Norway (95% CI for the difference 1.06–1.60), whereas the differences for Estonia versus Finland and for Norway versus Denmark were statistically non-significant (95% CI for the differences 0.82–1.37 and 0.81–1.23 respectively). The relatively large CI were due to the inclusion of standard error for the results of quality control in the calculations.

With regard to the frequencies of motile sperm and morphologically normal sperm, no effect of any of the confounders was apparent. Both parameters differed between the centers ($P < 0.005$). For motility, no quality control data were available, while all morphology smears were assessed in a blinded way by one person. Estimates of the frequencies of motile sperm, frequencies of normal sperm and the corresponding 95% CI were also calculated by regression analysis in order to present the results in Table 7 in a similar way as the other semen parameters. The men from Estonia were found to have the highest percentage of motile sperm, followed by the men from Denmark, Finland and Norway. The men from Estonia and Finland showed the highest frequency of morphologically normal sperm. Table 7 also presents the total number of motile and morphologically normal sperm. The highest values were detected among the men from Estonia and Finland. These figures were calculated from the total number of sperm and from the frequencies of motile and normal sperm, respectively.

Table 7. Semen parameters of young men from four Northern European cities

	Observed		Adjusted			
	All men		All men		Subgroup	
	Mean (SD)	Median (5–95)	Median	95% CI	Median	95% CI
Semen volume (mL)						
Estonia	3.2 (1.5)	3.1 (1.1–5.9)	3.0	2.8–3.3	3.0	2.7–3.4
Finland	3.3 (1.4)	3.0 (1.3–5.7)	3.4	3.2–3.6	3.3	3.0–3.6
Norway	3.1 (1.4)	2.9 (1.0–5.6)	3.1	3.0–3.5	3.2	3.0–3.6
Denmark	3.3 (1.5)	3.0 (1.3–5.8)	3.3	3.1–3.6	3.2	3.0–3.5
Sperm concentration (million/mL)						
Estonia	72 (55)	62 (13–175)	57	46–72	63	48–83
Finland	72 (59)	61 (3–184)	54	45–65	53	43–65
Norway	69 (56)	53 (7–179)	41	34–51	42	34–52
Denmark	57 (52)	44 (4–163)	41	35–48	45	37–54
Total sperm count (million)						
Estonia	235 (208)	180 (20–644)	174	137–222	192	144–255
Finland	221 (176)	194 (11–519)	185	154–223	176	141–218
Norway	205 (179)	158 (19–593)	133	108–164	136	109–171
Denmark	173 (162)	130 (9–506)	138	117–162	144	119–176
Motile sperm (%)						
Estonia	73 (11)	75 (57–86)	74[a] /128[b]	72–76	74[a]/142[b]	72–77
Finland	64 (13)	66 (42–80)	65[a] /120[b]	63–66	65[a]/114[b]	63–67
Norway	64 (9)	66 (45–76)	64[a] /85[b]	62–66	65[a]/88[b]	63–67
Denmark	65 (12)	68 (44–81)	66[a] /91[b]	64–67	66[a]/95[b]	64–67
Normal morphology (%)						
Estonia	9.2 (5.4)	9.0 (1.0–19.0)	9.2[a] /16[b]	8.1–10.2	9.2[a]/18[b]	8.0–10.5
Finland	8.9 (5.7)	9.0 (1.0–19.0)	8.9[a] /16[b]	8.4–9.5	8.8[a]/15[b]	8.0–9.5
Norway	6.9 (4.6)	7.0 (0.4–15.0)	6.9[a] /9[b]	6.2–7.6	7.0[a]/10[b]	6.1–7.8
Denmark	6.4 (4.9)	5.0 (1.0–16.0)	6.4[a] /9[b]	5.8–7.0	6.5[a]/9[b]	5.7–7.2

Observed: The results are based on raw data. SD = Standard deviation; 5–95 = 5th–95th percentile.

Adjusted: Median and 95% CI (5–95% confidence interval) calculated for the individual centers by linear regression analysis. Sperm concentration and total sperm count are adjusted to the determination level of the Danish laboratory (according to the results of quality control) and adjusted to the period of abstinence from ejaculation of at least 96 h. No effect of confounders was detected on motility and morphology. However, the results of regression analysis for these parameters are shown as 'adjusted' to indicate that the confounders had been investigated. Morphology smears were all assessed in Finland by Antero Horte

All men: The results based on all men participating in the study.

Subgroup: The results based entirely on the men not taking any medication, and on the men without any previous or current andrological diseases including known fertility problems. Information obtained from a self-administered questionnaire.

[a]The percentages of motile sperm or the percentages of morphologically normal sperm.

[b]The total number of motile or morphologically normal sperm per ejaculate (percentages x total sperm count). The adjustment to the Danish level of determination is based on the adjustment of total sperm counts.

Hour of day of blood sampling had a statistically significant effect as a confounder on testosterone (P = 0.02) with the highest hormone levels in the

morning hours, however in contrast with the Estonian-Lithuanian study, the effect of hour of day of blood sampling was non-significant ($P = 0.059$) for Inhibin-B, although the highest levels of Inhibin-B were recorded in the morning hours. Considering these results and those of previous publications finding a diurnal rhythm in the serum levels of reproductive hormones (Carlsen et al., 1999, and references therein), hour of day of blood sampling was included as a confounder in the further analysis of the hormone values. None of the other investigated possible confounders had any effect on the hormone parameters. The observed and the adjusted results of hormone assessments are summarized in Table 8. The men from Finland had the highest level of Inhibin-B, while the men from Estonia had the lowest level. In all four groups, regression coefficients were significantly positive for Inhibin-B ($P < 0.0005$) and negative for FSH ($P < 0.0005$) when included in a regression model of sperm concentration and total sperm count. The effects of increasing Inhibin-B and decreasing FSH were not statistically different between the centers. The men from Finland had the highest levels of testosterone, while the Estonian men had the second highest level of this hormone.

Table 8. Serum hormone parameters of young men from four Northern European cities

	Observed		Adjusted			
	All men		All men		Subgroup	
	Mean (SD)	Median (5–95)	Median	95% CI	Median	95% CI
FSH (IU/I)						
Estonia	3.8 (2.4)	3.2 (1.4–8.2)	3.3	3.0–3.7	2.9	2.5–3.4
Finland	3.9 (2.6)	3.3 (1.5–8.0)	3.4	3.1–3.6	3.3	3.0–3.6
Norway	3.9 (2.1)	3.5 (1.6–7.8)	3.4	3.2–3.7	3.4	3.2–3.7
Denmark	3.6 (2.4)	3.0 (1.2–8.1)	3.1	2.9–3.3	2.9	2.7–3.1
Inhibin-B (pg/mL)						
Estonia	204 (76)	194 (103–368)	198	180–217	196	175–220
Finland	245 (89)	234 (122–401)	228	216–241	230	214–246
Norway	221 (75)	214 (108–366)	208	197–220	206	193–220
Denmark	215 (79)	207 (97–346)	200	190–211	207	194–220
Testosterone (nmol/L)						
Estonia	25 (7)	25 (12–37)	24	23–25	23	22–25
Finland	26 (7)	26 (16–38)	26	25–27	25	24–27
Norway	23 (6)	23 (14–32)	22	21–23	22	21–23
Denmark	25 (6)	24 (16–35)	24	23–25	24	23–25

All serum samples were assessed in one laboratory.
Observed: The results are based on raw data. SD = Standard deviation; 5–95 = 5th–95th percentile.
Adjusted: The estimates and the 95% confidence interval (CI) calculated for individual centers by linear regression analysis. In assessment, hour of day of blood sampling is taken into account and adjusted to sampling carried out between 10:00 and 11:00 a.m..
All men: The results based on all men participating in the study.
Subgroup: The results based entirely on the men not taking any medication, and on the men without any previous or current andrological diseases including known fertility. Information obtained from a self-administered questionnaire.

The factors of possible confounding influence differed significantly between the three investigated groups but only duration of abstinence was shown to possess a confounding influence on semen volume, sperm concentration and total sperm count, as stated previously, and hour of the day of blood sampling was shown to have an effect on a majority of hormones.

Self-reported, previous medical conditions of the young men from the four countries are summarized in Table 9. The Estonian men reported most frequently having been diagnosed varicocele (7.8%) and inflammatory genital diseases as mumpsorchitis and gonorrhea. Less than 1% of the Finnish and Norwegian men had been treated for cryptorchidism, whereas 3.7% of the Danish men and 3.9% of the Estonian men had been treated for this condition.

Physical examination showed that a majority of the men had reached an adult level regarding the Tanner stage of pubic hair, although regional differences were apparent: Estonia 89.8%, Finland 99.7%, Norway 77.8% and Denmark 100% ($P < 0.0005$ for the difference between the groups). The men who had not reached Tanner stage 5 regarding pubic hair (adult level) had all stage 4.

Median testicular volumes (left/right testicles) were: Estonia 22/23 ml, Finland 20/23 ml, Denmark 18/20 ml and Norway 15/15 ml ($P < 0.0005$ for the difference between the groups). Regression coefficients were significantly positive for mean testis size when included in a regression model of sperm concentration, total sperm count and percentage of morphologically normal sperm (all $P < 0.0005$). The effect of a relative increase in testis size was similar for all centers (sperm concentration, total sperm count and frequency of morphologically normal sperm; all P = NS).

Table 9. Self-reported, conditions of young men from the four Northern European countries obtained from a self-administered questionnaire. The results are presented as the frequencies (%) of the number of men (*n*)

	Estonia (*n* = 104)	Finland (*n* = 324)	Norway (*n* = 240)	Denmark (*n* = 300)	*P* (between groups)*
Been diagnosed as having[a]:					
Varicocele	7.8	2.0	0.0	0.3	< 0.0005
Epididymitis	0.0	0.7	0.0	0.3	0.25 (NS)
Gonorrhoea	1.1	0.0	0.0	0.0	0.001
Chlamydia	2.3	1.3	0.4	1.4	0.13 (NS)
Orchitis due to parotitis	1.0	0.0	0.0	0.0	< 0.005
Cystitis	2.3	2.3	0.4	1.0	0.08 NS
Diabetes	0.0	0.0	0.0	0.0	1.0 (NS)
Thyroid Disease	0.0	0.3	0.4	0.0	0.10 (NS)
Been operated or treated for[b]:					
Varicocele	2.3	1.6	0.0	0.0	0.07 NS
Testicular torsion	2.3	1.0	0.0	0.7	0.26 (NS)
Testicular cancer	0.0	0.0	0.0	0.0	1.0 (NS)
Cryptorchidism	3.9	0.9	0.8	3.7	0.046
Other diseases of penis, urethra or scrotum	4.6	2.3	0.5	4.4	0.16 (NS)
Inguinal hernia	2.1	6.5	6.1	4.1	NS
Subject has:					
Had cryptorchidism, spontaneously descended[c]	6.7	9.9	4.6	5.0	0.04
Experienced fertility problems[d]	11.1	0.7	1.9	1.0	<0.0005
Caused a pregnancy[e]	6.1	2.4	6.3	3.4	0.11 (NS)
Taken any medication during the last 3 months[f]	4.9	16.4	13.9	16.7	0.02
Subgroup of men not affected by any of above conditions[g]	66.3	63.0	71.3	65.3	0.23 (NS)

[a]Questions were phrased, for example, as 'Have you ever been diagnosed by a doctor as having varicocele?' No information about how the diagnoses were obtained.
[b]Questions were phrased, for example, as 'Have you ever been operated for varicocele?' No further specific information regarding the type of treatment were available. For cryptorchidism, treatment could be either operation, hormonal treatment, or a combination of these.
[c]The question was 'Were you born with one or both of your testicles outside the scrotum but they descended spontaneously (without surgical or hormonal treatment)?'
[d]The question was 'Have you ever had regular intercourse without use of contraception for at least one year, without your partner becoming pregnant?'
[e]The question was 'Have you ever caused a pregnancy?'
[f]The question was 'Have you taken any medication during the last 3 months (before participating in this study)?'
[g]Men responding negatively to all categories of self-reported information.
*Pearson χ^2-test.

3. Prevalence of genital diseases and their influence on testis volume in the Estonian men (Paper 4)

Mean testis volumes (left/right testicles) were 20.3/21.3 ml in the infertile men (study group) and 21.7/22.4 ml in the unselected young men (control group) (P<0.001 for the difference between the groups).

The results of the prevalence of genital diseases, combining information from genital examination and clinical anamnesis, are summarized in Table 10. The prevalence of varicocele testis was higher in the study group than in the control group (37.5% vs. 24.1%, p<0.001). The prevalence of cryptorchidism was 3% in the study group and 1.7% in the control group (p=0.114). A substantial proportion (17% in the study group and 9% in the control group) of cryptorchid testes were untreated.

Similar proportions of the cases of testis cancer, hypospadia, hernia and hydrocele were found in both groups. The pathology of epididymis was significantly more prevalent in the group of the infertile men. A significantly higher proportion of men had suffered from orchitis, mumpsorchitis and severe testis trauma in the infertile men group compared to the control group.

Table 10. Prevalence of genital diseases in the infertile and control men in Estonia

Disease	Infertile men (n= 779) n (%)	Control group (n= 664) n (%)	P value (between groups)
Varicocele (total)[a,b,c]	291 (37.4)	160 (24.1)	<0.001
Left grade I[a]	99 (12.7)	68 (10.2)	0.140
Left grade II[a]	127 (16.3)	48 (7.2)	<0.001
Left grade III[a]	43 (5.5)	31 (4.7)	0.458
Cryptorchidism[a,b]	23 (3)	11 (1.7)	0.114
Epididymal pathology[a]	40 (5.1)	16 (2.5)	0.006
Orchitis, epididymitis[b]	18 (2.3)	1 (0.2)	<0.001
Mumpsorchitis[b]	8 (1)	1 (0.2)	0.035
Inguinal hernia[a]	2 (0.3)	2 (0.3)	0.875
Inguinal hernia, operated[a,b]	14 (1.8)	2 (0.3)	0.007
Testicular tumor[a]	2 (0.3)	2 (0.3)	0.875
Hypospadia[a]	4 (0.5)	4 (0.6)	0.824
Testicular trauma[b]	15 (1.9)	1 (0.2)	0.001
Hydrocele[a]	4 (0.5)	3 (0.5)	0.864

[a] clinical examination; [b] interview;
[c] varicocele (total) = left varicocele grade I; II and III + bilateral varicocele + left side varicocele + operated varicoceles

Table 11 shows the effect of different stages of varicocele testis on testis volume. Only in the group of the infertile men there was detected a negative influence of grade 1 varicocele on left testis volume. The right testis was not affected in either study group. The negative influence of grade II and grade III varicoceles on the volume of both testes was significant in the infertility group and slightly less pronounced in the control group.

Table 11. The effect of different stages of varicocele testis on testis volume.

Varicocele	Group	Number of subjects	Testis volume (ml)				P value (in comparison with same group of men without varicocele)	
			Left		Right		Left	Right
			Mean (SD)	Median (5–95)	Mean (SD)	Median (5–95)		
Left side Grade I	Control	68	21.7 (4.8)	22 (14–27)	23.2 (4.8)	23 (16–30)	0.382	0.315
	Infertile	99	20.5 (3.6)	20 (13–30)	21.4 (3.4)	22 (12–30)	**0.037**	0.201
Left side Grade II	Control	48	20.2 (4.1)	20 (11–30)	21.3 (4.1)	21 (15–28)	**0.060**	**0.045**
	Infertile	127	18.5 (4.2)	18 (5–30)	20.0 (4.4)	20 (5–32)	**<0.001**	**<0.001**
Left side Grade III	Control	30	19.0 (4.7)	18 (11–40)	21.5 (4.8)	21.5 (13–40)	**<0.001**	0.069
	Infertile	43	16.9 (4.3)	17 (8–24)	18.7 (5.2)	19 (2–30)	**<0.001**	**<0.001**
Bilateral	Control	10	20.8 (4.1)	22 (13–25)	21.4 (3.3)	22 (15–25)	0.554	0.522
	Infertile	14	18.5 (4.3)	18 (12–27)	19.4 (3.4)	19 (14–27)	**0.021**	**0.021**
Right side	Control	1	25.0	25	23.0	23	0.290	0.828
	Infertile	2	14.5 (14.8)	14.5 (4–27)	23 (1.4)	23 (22–24)	0.701	0.688
Operated	Control	1	17.0	17	18.0	18	0.151	0.208
	Infertile	6	19.7 (9.7)	18.5 (15–25)	21.3 (3.7)	19.5 (18–27)	0.398	0.671
Without varicocele	Control	506	22.1 (4.3)	22 (9–40)	22.5 (4.3)	23 (7–40)		
	Infertile	488	21.2 (6.0)	22 (0–50)	21.8 (6.1)	22 (0–50)		

4. Causes of male infertility in Estonia.

Table 12 summarizes the reproductive history of the infertile men investigated. Taken all groups together 35.5% of the men had caused pregnancies previously. Of the men 79% visited the andrologist due to primary infertility.

Table 13 summarizes the prevalence proportion of the diseases affecting potentially the genital organs as detected during a medical interview or during clinical and laboratory examinations.

There was no detectable difference in the prevalence of sexually transmitted diseases and prostatitis in the case history among different sperm count groups. However, there were more subjects in the azoospermia group (5.5%) with a history of orchitis and/or epididymitis. Rare causes accounting for single cases of severe forms of sperm pathology were misuse of anabolic steroids, chemotherapy due to malignancies, earlier vasectomy, testis cancer and disorders of development of the testes and prostate.

Of the infertile men 2% suffered from permanent sexual dysfunction. There were more sexual disorders in the cryptozoospermia group (15%) and among the men not delivering semen samples (6.2%) compared to the control group.

The prevalence of cryptorhidism was higher in all groups with a severe sperm pathology compared to the control group. An important fact was that in 10.4% of the cases cryptorhism had been left untreated. The prevalence of varicocele was equally high in all study groups but was statistically more often present in oligozoospermic men.

Although only 56.9% of the men in this study group passed tests for semen leukocytes, the prevalence of leukocytospermia was high in all oligozoospermia groups but also among men with normal sperm count.

Genetic pathology (CF mutations, Y chromosome microdeletions and chromosome abnormalities) was an important cause of male infertility in the groups of azoospemia and severe oligozoospermia (sperm count up to 5 mill/ml).

Besides the data presented in Table 13, we found that 38.9% of the men in the study group were smokers. The methods used for data collection and statistical analysis in our study did not detect a significant influence of current smoking on sperm count.

Table 12. Reproductive history of the infertile men with different grades of severity of their sperm pathology.

	Azoo-spermia n=109	Crypto-zoospermia n=20	0.5–5 mill/ml n=92	5.1–10 mill/ml n=77	10.1–20 mill/ml n=174	20.1–40 mill/ml n=303	>40 mill/ml (control group) n=617	Did not deliver semen sample N=145
Man has caused pregnancies	**13 (11.9%)[1]**	**3 (15.0%)[1]**	**21 (22.8%)[1]**	**21 (27.3%)[1]**	**57 (32.8%)[1]**	**100 (33.0%)[1]**	256 (41.8%)	74 (51.0%)
Primary infertility	**96 (88.1%)[1]**	**19 (95.0%)[1]**	78 (84.8%)	63 (81.8%)	135 (77.6%)	**252 (83.2%)[1]**	468 (75.9%)	**98 (67.6%)[1]**
Habitual abortions		1 (5%)			1 (0.6%)	2 (0.7%)	18 (2.9%)	4 (2.8%)

[1] $p<0.05$ in comparison with control group

Table 13. Prevalence proportion of the diseases affecting the genital organs as detected during a medical interview or during clinical and laboratory examinations.

	Azoo-spermia n=109	Crypto-zoospermia n=20	0.5–5 mill/ml n=92	5.1–10 mill/ml n=77	10.1–20 mill/ml n=174	20.1–40 mill/ml n=303	>40 mill/ml (control group) n=617	Did not deliver semen sample n=145
Sexually transmitted diseases	18 (16.8%)	6 (30%)	19 (20.7%)	19 (24.7%)	58 (33.5%)	97 (32.1%)	202 (32.9%)	38 (26.2%)
Orchitis, epididymitis	**6 (5.5%)[1]**	0	2 (2.2%)	3 (3.9%)	4 (2.3%)	9 (3%)	8 (1.4%)	1 (0.7%)
Prostatitis	0	0	2 (2.2%)	2 (2.6%)	5 (2.9%)	12 (4%)	24 (3.9%)	7 (4.8%)
Mumpsorchitis	0	0	2 (2.2%)	**3 (3.9%)[1]**	2 (1.1%)	6 (2%)	5 (0.8%)	0
Testis traumas	**5 (4.6%)[1]**	0	0	**4 (5.2%)[1]**	3 (1.7%)	9 (3%)	9 (1.5%)	2 (1.4%)
Sexual dysfunctions	3 (2.9%)	**3 (15%)[1]**	2 (2.2%)	1 (1.4%)	2 (1.1%)	3 (1.1%)	8 (1.4%)	**9 (6.2%)[1]**
Cryptorchidism	**12 (11%)[1]**	**2 (10%)[1]**	**8 (8.7%)[1]**	**3 (3.9%)[1]**	5 (2.9%)	7 (2.3%)	7 (1.1%)	4 (2.8%)
-unilateral	4 (3.7%)	2 (10%)	5 (5.6%)	1 (1.3%)	2 (1.1%)	4 (1.3%)	6 (1%)	1 (0.7%)
-bilateral	7 (6.7%)	0	3 (3.3%)	0	2 (1.1%)	0	0	1 (0.7%)
-fixed to the external orifice of inguinal canal	1 (1.0%)	0	0	2 (2.6%)	1 (0.6%)	3 (1.1%)	1 (0.2%)	2 (1.4%)
Not treated	3	0	0	2	1	5	2	2

Table 13. Continuation

	Azoo-spermia n=109	Crypto-zoospermia n=20	0.5–5 mill/ml n=92	5.1–10 mill/ml n=77	10.1–20 mill/ml n=174	20.1–40 mill/ml n=303	>40 mill/ml (control group) n=617	Did not deliver semen sample n=145
Varicocele	28 (26.9%)	4 (20%)	**53 (58.9%)[1]**	**37 (50%)[1]**	**74 (44.3%)[1]**	**116 (41.3%)[1]**	191 (32.3%)	47 (32.6%)
– left grade I	7 (6.7%)	0	11 (12.2%)	13 (17.6%)	19 (11.4%)	35 (12.5%)	80 (13.5%)	15 (10.4%)
– left grade II	15 (14.4%)	2 (10%)	**27 (30.0%)[1]**	11 (14.9%)	**42 (25.2%)[1]**	**56 (19.9%)[1]**	81 (13.7%)	24 (16.7%)
– left grade III	2 (1.9%)	1 (5%)	**10 (11.1%)[1]**	**7 (9.5%)[1]**	8 (4.8%)	**19 (6.8%)[1]**	16 (2.7%)	7 (4.9%)
– bilateral	3 (2.9%)	1 (5%)	**5 (5.6%)[1]**	**5 (6.8%)[1]**	3 (1.8%)	3 (1.1%)	11 (1.9%)	1 (0.7%)
– right-sided	0	0	0	1 (1.4%)	1 (0.6%)	1 (0.4%)	2 (0.3%)	0
– operated	1 (1.0%)	0	0	0	1 (0.6%)	2 (0.7%)	1 (0.2%)	0
Epididymal pathology	**19 (17.9) [1]**	2 (10%)	2 (2.2%)	4 (5.4%)	5 (3%)	8 (2.8%)	20 (3.4%)	5 (3.5%)
– thickening	**19 (17.9) [1]**	1 (5%)	0	1 (1.4%)	5 (3%)	3 (1.1%)	5 (0.8%)	3 (2.1%)
– cysts	0	1 (5%)	2 (2.2%)	3 (4.1%)	0	5 (1.8%)	15 (2.5)	2 (1.4%)
Inguinal hernia	4 (3.7%)	0	3 (3.3%)	**4 (5.2%)[1]**	5 (2.9%)	9 (3%)	11 (1.8%)	2 (1.4%)
- operated in childhood	2 (1.8%)	0	1 (1.1%)	**3 (3.9%)[1]**	2 (1.1%)	1 (0.3%)	4 (0.6%)	0
- operated in adulthood	2 (1.8%)	0	1 (1.1%)	0	3 (1.7%)	6 (2%)	6 (1%)	2 (1.4%)
- detected	0	0	1 (1.1%)	1 (1.3%)	0	2 (0.7%)	1 (0.2%)	0
Leukocytospermia	4 (6.7%)	1 (7.1%)	**14 (25.5%)[1]**	9 (22%)	17 (17.5%)	30 (14.1%)	56 (13.9%)	0
Genetic causes	**14 (13.2%)[1]**	0	**3 (3.3%)[1]**	0	0	1 (0.3%)	0	0
Hypogonadism	1 (1.0%)	0	0	0	1 (0.6%)	0	1 (0.2%)	1 (0.7%)

[1] $p<0.05$ in comparison with the control group

V. DISCUSSION

1. Quality control

Standardization of the methods of semen analysis and training of the technicians performing semen analysis thorough the study in Tartu but also technicians in the laboratories of the other international partners participating in this study, started with ESHRE-NAFA Basic Semen Analysis Workshop in Stockholm, 1995. In the frame of the study of military recruits the meeting on standardization of semen analysis before the start of the study ensured that all participating laboratories used the same methods for semen analysis. The program of the external quality control lasting throughout the study allowed to calibrate the determination levels of different laboratories to a comparable level. The results of sperm concentrations in our studies on the reproductive function of young men are presented first as raw data and thereafter as adjusted values where both inter-laboratory differences and major a confounder of this semen parameter, period of abstinence, were taken into account. The Danish laboratory was chosen as the reference laboratory in order to make the results as comparable as possible to the results of previous publications including the results from the Danish laboratory (Bonde et al., 1998; Andersen et al., 2000; Jørgensen et al., 2001).

In our study on standardization of andrological clinical examination we found highly significant differences in the results between the investigators/ andrologists from the centers participating in the coordinated Nordic-Baltic study on the reproductive function of male military recruits. Detected inter-investigator differences in measurement of testicular size and other genital pathologies may significantly influence the interpretation of the results of multicenter male fertility studies. Therefore the results about testis volume within the current study are not directly comparable and the rest of the data about genital pathologies collected during the study were left out of publications as disconcerting. In further research our experience should be taken into account as there are real possibilities to reduce these differences in clinical examination by using common training and proper standardization of the protocols of andrological examination prior to studies. As in Estonia all clinical examinations were performed by one experienced examiner the major source of error was avoided.

2. Reproductive function of young males in Estonia in comparison with the Nordic-Baltic area

In this coordinated cross-sectional study we investigated the quantitative and qualitative semen parameters in five cities in the Nordic-Baltic area: Tartu in Estonia, Kaunas in Lithuania, Turku in Finland, Oslo in Norway and Copenhagen in Denmark. The studied Estonian, Finnish and Lithuanian men had higher sperm concentration and higher frequency of morphologically normal sperm than the Norwegian and Danish men. The highest sperm concentration and the highest frequency of morphologically normal spermatozoa were detected in the group of Estonian soldiers. To serve in the army, men should have a good health condition in general, without any serious chronic diseases or serious mental or physical disabilities. Hence soldiers may have a better general health condition than conscripts from general population and this better health condition may be reflected in semen parameters, too.

The men from Estonian and Lithuanian general population studied in the Paper 2 were 1–2 years older at the time of investigation than the men from Denmark, Norway, Finland and Estonia studied in the Paper 3. The results of these papers are therefore not directly comparable. However, statistical analyses were carried out in the same way and sperm concentrations and total sperm counts were adjusted to the level of the reference laboratory in order to make the results as comparable as possible. The statistical difference in age was also shown between the groups compared in both papers. From the biological point of view, we regard these ages as rather close. Additionally, in statistical analysis we failed to show any confounding influence of age on the semen and hormonal parameters. Thus, the differences in ages between the groups are not likely to explain our findings. Year of birth was also investigated as a confounder and was found to exert no influence on these parameters.

Participation rate among the invited men from the general population in Estonia, but also among the men at other study centers, was rather low as is almost always the case when delivery of semen samples is requested. The participants were included when they showed up for the compulsory medical examination irrespective of the outcome of this examination. Thus, they were not selected because of diseases, regular medications or their fertility status and, therefore, we have no reason to doubt that the men included in these two groups were representatives of the normal population. The financial compensation received for participating in the study was unlikely to have led to the selection of men with reduced semen quality. In fact, if compensation had not been provided it is more likely that men suffering from some kind of disease would have been more interested in participation – hoping to receive advice – than men without diseases.

Previously, there has been made one attempt to control selection bias in studies on young men – male military recruits. Andersen (2000) included into her study a control group with a less demanding participation protocol, which increased the participation rate up to 79%. The idea was to control selection bias via comparison of reproductive hormones as surrogate markers of reproductive health. The subjects of the control group were enrolled according to a common protocol when they reported for their medical examination at the military health board. The only request for this control group was a blood sample. The subjects received financial contribution (30 EUR compared to 60 EUR to the participants of the full study) for their participation. When the results of the two groups (participants of the full study and hormone only group) were compared, no significant differences were observed in the concentration of FSH, Inhibin-B or any other reproductive hormones (Andersen, 2000).

In contrast to general population the participation rate among the Estonian soldiers was much higher. We can only speculate what caused this difference in the participation rates. The soldiers received exactly the same economic compensation as the men from the general population, but in the group of soldiers compensation may have been a stronger motivation for participation. The men of the study lived in a closed military setting and only received a quite small salary, whereas majority of the men from the general population lived with their families and had the opportunity to earn money with ordinary work.

We believe that the results of sperm count and morphology are not likely to be explained by technical bias. Sperm concentration was controlled by an external quality control program and the presented adjusted values take into account, besides to period of abstinence, also inter-laboratory differences. Furthermore, assessment of morphology smears was centralized and carried out by one investigator in Turku, Finland.

The present results of semen analysis are of interest regarding the reported incidences of testicular cancer in Estonia, Lithuania, Finland, Norway and Denmark, as they indicate an east–west gradient in the semen parameters in parallel to the gradient in the incidence of testicular cancer in these countries. It means that the best semen quality was detected in the areas with the lowest risk of testicular cancer (age-standardized incidence rate per 10^5 in Lithuania is 1.6, in Estonia 2.2, and in Finland 3.2) and the poorest semen quality was noted in the Danish and Norwegian groups who were at the highest risk for testicular cancer (incidence rates in Denmark and Norway are 10.8 and 11.0 per 10^5 , respectively) (International Agency for Research on Cancer. Globocan 2002). Thus, the present findings tend to support to the hypothesis that testicular cancer and impaired spermatogenesis may sometimes share common etiological factors.

Although low, the incidence rates of testicular cancer have been increasing in Estonia, Lithuania and Finland during recent years (Adami et al., 1994; Ferlay et al., 2001). The question is then whether the frequency of men with poor

quality is also increasing in these countries. There seem to be insufficient retrospective data to answer the question with certainty. However, sperm count for Finnish men was lower than could be expected from previously published studies. Two publications (Suominen and Vierula, 1993; Vierula et al., 1996) have reported that Finnish men had high unchanged mean sperm concentrations of 94–114 mill/ml during the period 1958–1992. The differences between these data and the results of the present study are impressive. Sperm concentrations were, in principle, assessed according to the same methods in these two retrospective studies and in the present study. However, the validity of such comparisons is always hampered by the fact that they are based on retrospective data and on study groups that are not completely comparable. As previously stated, only prospective studies will be able to indicate a possible time trend in semen qualities (Irvine et al, 1996). Unfortunately, no previous data are available to analyze the secular trend in sperm quality in Estonia. It is important to know that the superior sperm count, compared to that for our Baltic-Nordic neighbors, reported in our studies on Estonian men, is about twice below the level reported from the Western world 50 years ago and in Finland 10–40 years ago.

The relative difference in semen volume and hence also in total sperm count between the Estonian and the Lithuanian men, detected in Paper 2, is most likely the result of using different methods of assessment of volume. The Lithuanian laboratory assessed volume by aspiration into a pipette. This method is known to yield a lower values compared with values obtained by weighing samples (Jørgensen et al., 1997). We have no reliable data to indicate how much the use of different methods affected the semen volumes reported by the Lithuanian laboratory in comparison with the values reported by the Estonian laboratory. However, the paper by Jørgensen et al. (1997) indicates that aspiration resulted in 0.3 or 0.5 mL lower values of median semen volumes compared with the corresponding values obtained by weighing. This difference is approximately the same as the difference in the observed median semen volumes between the Lithuanian and the Estonian study subjects. A cautious conclusion would therefore be that there exists no real difference between the three Baltic groups regarding semen volume. As a consequence of this, the magnitude of the detected difference between the Lithuanian and the Estonian men, regarding total sperm counts may be smaller than is apparent from Table 4, but the difference does still exist because of the differences in sperm concentrations.

Like other studies (MacLeod and Gold, 1956; Jørgensen et al., 2001), we detected that ejaculation abstinence period was an important confounder of sperm count that should be taken into account. Apparently, sperm concentration and total sperm count increase up to 4 days of abstinence (96 h). The WHO recommends an ejaculation abstinence period of at least 48 h (World Health Organization, 1992). Our results indicate that this lower limit should be

increased to 96 h, as in another recent publication (Jørgensen et al., 2001), although neither of the studies was designed to answer this question. Additionally, the upper limit of 7 days recommended by the WHO may also be questioned as there was found no effect of abstinence period longer than 7 days. Magnus and coworkers (1991) have shown that in men with low sperm counts the frequency of progressive motile spermatozoa and the total number of spermatozoa may be increasing if the duration of abstinence is as long as 6–10 days.

The results of the hormone analyses in our study were in many aspects quite surprising. Inhibin-B is considered to reflect feedback information from the testicle to the pituitary gland regarding sperm production (Jensen et al., 1997). Within all five groups of men, correlation was found between the increasing levels of Inhibin-B and increasing sperm counts. However, proceeding from the results of sperm concentration and total sperm counts, it could be expected that the levels of Inhibin-B would be the highest in the Estonian groups, which, however was not the case. The highest Inhibin-B level was detected among the Finnish and the Lithuanian men also belonging to the group of high sperm concentration and total sperm count as well. In contrast, the Estonian and the Danish men had almost similar Inhibin-B levels that were lower than those of the Norwegian men, despite the fact that the Estonian men had higher sperm counts. Completely different results were obtained from the study group of Estonian soldiers who had the highest sperm counts but the lowest Inhibin-B level. It can be cautiously concluded that many aspects of Inhibin-B have not yet been characterized completely.

The Estonian soldiers also constitute a special group regarding testosterone because they had the lowest level of this marker among the three groups investigated in Paper 2. We have no information to clarify whether these low levels reflect intensive physical exercise or genuine differences. However, there is no doubt that the soldiers were physically active because of their military service.

Unfortunately, only at the end of the study it turned out that the collected results of clinical genital examination, except for the results of testis volume, were not comparable. Therefore, from among these results, only the data of testis volume were included in this study. The higher testicular volumes among the Lithuanian, Estonian and Finnish men compared with the Danish and Norwegian men, may have been in accordance with the higher sperm counts among these groups. However, the majority of differences in testicular volumes between countries were most likely caused by inter-observer variation as it was almost of the same magnitude as the difference between the study subjects from the four countries (Paper 1). In all five centers increasing sperm concentration, total counts and frequency of normal sperm corresponded with increasing mean testis size.

3. Prevalence of genital diseases and their influence on testis volume in Estonian men

This is probably the first study in which both the study group and the control group were examined by one investigator in the same time window.

In the clinical examination we detected that, as expected, the testis volume of the infertile men was significantly lower than that of the subjects in the control group and that the subjects from the infertility group harbored more often than subjects from the general population genital diseases or conditions like varicocele testis, epididymal pathology and operated inguinal hernias. The patients' history showed that the subjects in the infertility group had more often orchitis/epididymitis, mumpsorchitis and testicular traumas.

The prevalence of varicoceles in general population is estimated to be 15–20% but it is 30–40% among men attending infertility clinics (Jarow, 2001). In our study the prevalence of varicocele testis in the infertile men (37.4%) correlates with these data, while it was higher (24.1%) than expected in the control group. However, there are at least two previous studies where the relatively high prevalence of varicocele was detected in unselected cohorts. Pinto et al (1994) estimated the prevalence of varicocele testis at 23.4% in the group of men who visited the urology clinic due to the reasons other than male infertility. Thomason et al (1979) performed a similar study on military recruits and found that 30.7% of all recruits had a left varicocele, among them 14% were small and 16.7% moderate or large varicoceles. The latter figures are in good accordance with the varicocele stages established the present study. The most probable explanation for the above divergence is that detection of varicocele is dependent on the specialization and previous experience of examining doctors as became evident also in our Nordic-Baltic fertility studies (Paper 1). Moreover, it is likely that that the degree of care taken to detect varicocele may be different in different target groups (Jarow, 2001). Patients examined for infertility are usually examined very carefully for even small varicoceles, whereas, small or moderate-sized varicoceles are likely overlooked during physical examination of school students or military recruits. Yet we can not entirely exclude the possibility that the high prevalence of varicocele testis in our study may indicate the true difference between Estonian men and men of other ethnicities.

In accordance with other investigators (Lipshults et al, 1977; Zini et al, 1997), we found a negative impact of varicocele on testis volume. Most investigators have used the right testis as the normal control when comparing the volume of the two testes. Therefore little attention has been paid to the influence of the left varicocele on the volume of the right testis. Our study clearly indicates a negative effect of the left varicocele on the volume of the left testis in both study and control groups. The negative effect of the left varicocele

on the volume of the right testis was significant in the group of the infertile men, but a borderline or a statistically non-significant trend was detected in the group of young men (control). Our finding is in accordance with a study of McFadden and Meehan (1978) who found that varicocele caused histological changes at first in the left testis and thereafter in the right one. On the contrary, Rolf et al (1992) noted that the influence of varicocele on fertility has developed by the age of puberty and did not progress later in life. This controversy can arise from the fact that their study is based solely on the data of sperm analysis. It has been shown also by other investigators (Hans et al, 1991; Paducah et al, 1996) that varicocele has a time dependent increasing negative effect on testis volume and sperm quality.

Cryptorchidism is one of the most important pathologies that deteriorate male fertility. Unfortunately, so far no epidemiological study has been conducted on this pathology in Estonia. However, the prevalence of cryptorchidism in our control group is in accordance with the results of recent epidemiological studies in our neighboring countries Lithuania and Finland (Preiksa et al, 2005; Boisen et al, 2004). The alarming fact, indicating shortages in the medical system of Estonia, is that a significant number of cryptorhidism cases are diagnosed and/or treated too late or remain untreated altogether.

The higher prevalence of epididymal pathology, orchitis, mumpsorchitis and testis trauma in the group of the infertile men points to the known negative impact of these diseases on male infertility, at the same times we can not exclude some possible cumulative effect with increasing age, either.

4. Causes of male infertility in Estonia.

In this study we analyzed the data of 1537 infertile men regarding their reproductive history, major lifestyle factors, general and genital tract diseases and the results of their genital examination. We found that the most prevalent risk factors in the azoospermia group were genetic defects, epididymal pathology, orchitis/epididymitis, genital traumas and cryptorhidism. The risk factors for severe oligozoospermia were genetic defects, cryptorchidism, varicocele and leukocytospermia. The risk factors for oligozoospermia were varicocele, cryptorchidism, inguinal hernia operations, mumpsorchitis and genital traumas. Sexual dysfunctions were common in the cryptozoospemia group and often men with this kind of problems refused to pass proper tests as semen analysis.

In the interpretation of the current data we should admit the major weakness of the study: the collected data are based on clinical information, while not all men passed all necessary diagnostic steps to detect all potential causes of decreased sperm count.

The paucity of studies on the causes of male infertility and subfertility and the controversial results in published studies (WHO, 1987; Pierik et al, 2000;

Nieshlag, 2001) demonstrate the sophisticated nature of the topic. Still, for planning optimal infertility service and, even more important, for prevention of male factor infertility, we should know the magnitude and relative influence of different risk factors for the male reproductive tract.

As there do not exist any absolute criteria for distinguishing between fertile and infertile men, we tested the usefulness of grouping the subjects according to their count of spermatozoa in 1 ml of seminal fluid. Sperm count has shown the best correlation with male fertility potential in earlier studies. Bonde et al (1998) found that male fertility, measured as time to pregnancy, decreased significantly if the count of spermatozoa was less than 40 mill/ml. The next decline of male fertility occurred at the level of 20 mill/ml and then at 10 mill/ml. It was unlikely to achieve natural pregnancy at the count of spermatozoa less than 10 mill/ml. At the count of spermatozoa 5 mill/ml and less, genetic disorders served as important causes of sperm pathology. Such an arrangement of the study groups enabled us to assess the importance of different diseases and factors as the causes of sperm pathology with different grades of severity.

We can divide the causes and risk factors of male infertility into preventable, treatable and untreatable groups. A majority of the risk factors detected in our study were, in fact, preventable and treatable, indicating an urgent need for a better understanding of doctors and community about the role of these diseases in male reproductive health.

Undoubtedly, by improving the knowledge of sexual hygiene and minimizing risk behavior, it would be possible to reduce the incidence of orchitis and epididymitis as well as a number of severe testis traumas. As regards inflammatory genital diseases, an important preventable and treatable cause of infertility is leukocytospermia. Unfortunately, the test for measuring leukocytospermia was not available in all of the infertility laboratories serving our cohort and appropriate testing was performed in only 56.9% of our study subjects. In spite of the lower number of the tested men, leukocytospermia appeared to be one the most prevalent potential causes of decreased semen quality and hence of male infertility in Estonia.

Fertility of men is significantly dependent on the quality of pediatric surgery. Inguinal vasal obstruction related to inguinal herniorrhaphy is believed to be an uncommon cause of male infertility but the true incidence of inguinal vasal obstruction is unknown and probably underreported. It has been suggested that the incidence of vasal injury ranges from 0.3% to 7.2% (Shin et al., 2005) but Marsuda et al. (1992) investigated subfertile men with a history of hernia repair and found unilateral obstruction to be present even in 26.7% of cases. To cause azoospermia, there must occur either bilateral obstruction or unilateral obstruction with a nonfunctioning contralateral testis. Unilateral obstruction alone usually causes only decreased sperm concentration. In addition to the risk of obstructing the seminal ducts, there is also the risk of damaging blood vessels

and hence impairing testis blood supply during inguinal hernia operations. In our study we established inguinal hernia operation as a statistically significant risk factor in the group of oligozoospermia III, but a statistically non-significant trend for an increased rate of this risk factor was detectable in all oligozoospemia groups.

Cryptorchidism is widely accepted as one of the most important risk factors for severe sperm pathology and the results of our study support this finding. Although being not a preventable cause of male infertility the prognosis of future fertility of men with cryptorchid testicle(s) is significantly dependent on the timing and quality of corrective operations. Recent studies indicate that treatment between 6 and 18 months of age is recommended (Kass et al., 1996). Our study demonstrates that in Estonia the main problem is still early diagnosis and patient referral to pediatric urologists. In our study group none of the subjects with cryptorchidism was treated in above optimal time period for him and even 10.4% of the infertile men with cryptorchidism had not been treated altogether before presenting to the andrololologist due to a couple infertility problem.

Varicocele is one of the most controversial diseases among the factors contributing to male infertility (Jarow, 2001). It is the most frequent potentially treatable genital disease detected in infertile men. In our study we detected varicocele testis as the most prevalent risk factor in all oligozoospermia groups. Thus our data support existing evidence that varicocele is an important risk factor for testis damage and male infertility. Further studies should focus more on development of an optimal treatment strategy for this prevalent and important disease of the male reproductive system.

Genetic causes of male infertility are the only true non-preventable and non-treatable causes of male infertility. Still, we believe that it is important to find out genetic reasons in infertile men as this would help understand the nature of the pathology, prevent using ineffective invasive treatment methods, enable to predict the efficiency of laboratory treatment methods of infertility and provide a basis for genetic counseling for the genetic risks of possible IVF born babies. The prevalence of genetic pathologies in our study does not pretend to express the true prevalence of these pathologies in infertile Estonian men. Thorough large scale prospective studies of this entity will be published in 2006/2007.

VII. CONCLUSIONS

1. Inter-investigator differences in measurement of testicular size and genital pathologies may significantly influence the interpretation of the results of multicenter studies on male reproductive function. These differences should be eliminated by means of common training and standardization of andrological clinical examination prior to launching studies.
2. The Estonian – Lithuanin study and the Estonian – Nordic study on reproductive function revealed that median values of the major markers of male reproductive function were the following:
   2.1. Testis volume assessed with the Prader orchidometer was 22 ml (left testicle) and 23 ml (right testicle) in both studies.
   2.2. Sperm concentration was 67 and 57 mill/ml, the frequencies of morphologically normal spermatozoa were 7.7 and 9.2% and the percentages of motile spermatozoa were 75 and 74%, respectively.
   2.3. The blood levels of the reproductive hormone Inhibin-B was 220 and 198 pg/ml and that of FSH was 3.4 and 3.3 IU/l, respectively.
3. Semen quality and other markers of male reproductive function of Estonian young men are comparable to those of Finnish and Lithuanian men but are better than those of Norwegian and Danish men, revealing an east-west gradient in male reproductive function in the Nordic-Baltic area.
4. There is positive correlation between the levels of Inhibin-B and sperm counts. The results of an exceptional study group, Estonian soldiers with the highest sperm counts but the lowest Inhibin-B level, indicate the need for additional studies on the regulatory mechanisms of this hormone.
5. The prevalence of clinically detectable varicocele testis, epididymal pathology and a history of testicular traumas, epididymitis/orchitis and mumps-orchitis as well as the prevalence of operated inguinal hernias are statistically higher in the group of infertile men than in the control group, indicating the causal relationship of these diseases with male factor infertility in Estonia.
6. Varicocele testis is the most prevalent risk factor for male infertility in Estonia. Left-side varicocele exerts a grade dependent negative effect on the volume of both testicles.
7. As absolute criteria for differentiation between fertile and infertile men are lacking, grouping of men according to the count of spermatozoa can be suggested in epidemiological studies on the causes of male infertility. Such grouping allows to assess more precisely the relative importance of different pathologies and risk factors in male infertility.
8. A majority of the important causes of male infertility found in the study are treatable or even preventable conditions. Increase in the current awareness of the risk factors for male infertility among population as well as at different levels of the medical system will create better prerequisites for their prevention, early detection and timely treatment in the future.

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SUMMARY IN ESTONIAN

Meeste viljakus ja selle riskitegurid Eestis

Viljatuseks nimetatakse situatsiooni, kus vaatamata partnerite soovile ja regulaarsele seksuaalelule pole 12 kuu jooksul rasestumine õnnestunud. Viimaste aastate uuringud on näidanud, et 10–16% arenenud riikide peredel pole bioloogilistel põhjustel õnnestunud saada soovitud arvu lapsi. Umbes 60% juhtudel on üheks võimalikuks viljatuse põhjuseks häired mehe reproduktiivses tervises.

Viimase paarikümne aasta jooksul on viljatuse laboratoorsed ravivõimalused oluliselt avardunud. Negatiivse asjaoluna on uute ravimeetodite kättesaadavus oluliselt vähendanud huvi mehe viljakust kahjustavate haiguste ja tegurite analüüsi vastu. Mehepoolse viljatuse põhjuste kohta on avaldatud vaid üksikud uurimused ja nende tulemused on sageli olnud vastukäivad. Enamikes maailma kliinikutes hinnatakse mehe viljakust ainult sperma analüüsi alusel, pööramata tähelepanu teistele mehe reproduktiivse funktsiooni markeritele nagu munandi maht ja suguhormoonid. Enamasti jäävad välja selgitamata ka spermapatoloogia ja mehe viljatuse tegelikud põhjused.

Mitmed uuringud on näidanud, et viimase poole sajandi jooksul on arenenud riikides toimunud oluline sperma kvaliteedi langus. Geograafiliselt ja klimaatiliselt lähedastes piirkondades, nagu näiteks Taani ja Soome, on sperma kvaliteet osutunud väga erinevaks. Selleks, et täpsustada meeste viljakuse seisundit ja võimalusel jälgida ka muutuste dünaamikat, käivitas prof. Skakkebaeki poolt juhitav Kopenhaageni Ülikooli androloogide töörühm 1995. aastal mitmed koordineeritud prospektiivsed meeste reproduktiivse funktsiooni uuringud Põhja- ja Lääne Euroopas, kus üheks peamiseks uuritavate grupiks olid noored mehed – sõjaväekohuslased. Selle uuringuga liitus 1996. aastal ka Tartu Ülikooli Kliinikumi androloogia kabinet.

Eestis pole seni meeste reproduktiivse funktsiooni alal uuringuid läbi viidud. Puuduvad teadmised meeste reproduktiivse funktsiooni üldise seisundi kohta ja pole teada ka olulisemate mehepoolse viljatuse riskitegurite levimus Eestis.

Uurimistöö eesmärgid ja ülesanded

1. Täiustada meeste reproduktiivfunktsiooni alal mitmes keskuses üheaegselt teostatavates uuringutes kogutud kliiniliste andmete standardiseerimise meetodeid.
2. Uurida noorte eesti meeste reproduktiivse funktsiooni markereid: munandi mahtu, sperma kvaliteeti ja reproduktiivhormoonide taset veres.
3. Võrrelda eesti meeste reproduktiivse funktsiooni seisundit teiste Balti- ja Põhjamaade meeste vastavate näitajatega.

4. Välja selgitada olulisemate mehe reproduktiivset funktsiooni mõjutavate sugutrakti haiguste levimus eesti meestel.
5. Selgitada spermatosoidide arvul põhineva uuritavate grupeerimise informatiivsus mehepoolse viljatuse riskitegurite tuvastamisel.

Uuritav materjal ja meetodid

Uuringu esimeses faasis toimus sperma analüüsi meetodite standardiseerimise koolitus ja kogu uuringu toimumise aja osalesid kõik sperma analüüsi laborid igakuises spermatosoidide arvu määramise kvaliteedikontrolli programmis. Kvaliteedikontrolli tulemuste alusel tuvastati uuringus osalenud laborite vahelised erinevused ja selle alusel teostati spermatosoidide arvu statistiline korrektsioon tasemele nagu oleks analüüs teostatud ühes laboris. Alles uuringu lõppfaasis avastasime, et kogutud kliinilise läbivaatuse tulemused on raskesti võrreldavad ja arvatavate erinevuste kontrollimiseks viisime koheselt läbi kliinilise läbivaatuse standardiseerimise seminari, mille käigus osalevate töögruppide kliinilist läbivaatust teostavad arstid uurisid kahel päeval 23 erineva sugutrakti arengu ja genitaalpatoloogiaga Taani meest. Analüüs näitas, et uuringus osalenud arstide kliinilise läbivaatuse tulemused erinesid sedavõrd, et vastavad andmed, välja arvatud munandimaht, jäeti edasistest publikatsioonidest välja. Eestis teostas kõigi meeste läbivaatuse üks kogenud meestearst, mistõttu kogutud andmed on Eesti kontekstis kasutatavad.

Töö käigus uuriti kokku 2502 eesti meest. Töö esimeses osas, noorte meeste viljakuse uuringus, on analüüsitud 183 sõjaväekohuslase ja 118 kohustuslikus armeeteenistuses viibinud mehe reproduktiivfunktsiooni tähtsamaid näitajaid – sperma kvaliteeti, reproduktiivhormoonide taset veres, kliinilise läbivaatuse ja küsimustiku tulemusi. 664 meest, kes ei soovinud läbida täisuuringut, moodustasid kontrollrühma, kellel teostasime vaid kliinilise läbivaatuse. Eesti meeste reproduktiivse funktsiooni parameetreid on võrreldud Leedu, Soome, Norra ja Taani meeste vastavate andmetega, kusjuures meeste kaasamine uuringusse ja teostatud uuringud järgisid kõigis keskustes ühtset uuringuprotokolli.

Uuringu teises osas analüüsisime ajavahemikus 1997–2002 kahes Tartu kliinikus (Tartu Ülikooli Kliinikum ja Tähe Erakliinik) viljatuse probleemiga androloogi vastuvõtule pöördunud meeste sperma analüüsi ja kliinilise läbivaatuse andmeid. Uuritavate grupi moodustasid 1537 meest. Selleks, et vältida võimalikku ajas toimuvat muutust kliinilise läbivaatuse registreerimisel moodustasime uuritavate alamrühma, kelle uuringud kattusid ajaliselt noorte meeste viljakuse uuringus osalenud meeste uuringutega (uuritavate arv 779 meest). Alamrühma võrdlesime noorte meeste viljakuse uuringu kontrollrühmaga tuvastamaks rühmadevahelisi erisusi sugutrakti mõjutavate haiguste ja teiste mõjurite osas.

Selleks, et analüüsida erinevate sugutrakti haiguste ja genitaalpatoloogiate levimust erineva viljakuse prognoosiga spermatosoidide arvuga meestel, jagasime viljatuse uuringu üldgrupi alamrühmadeks vastavalt teadaolevale spermatosoidide arvu mõjule mehe viljakusele.

Uurimistööst tulenevad järeldused

1. Uurijatevahelised erinevused munandi mahu ja genitaalpatoloogiate tuvastamisel võivad olulisel määral mõjutada üheaegselt mitmes keskuses teostatavate mehe viljakust käsitlevate uuringute tulemuste interpretatsiooni. Need erinevused tuleks elimineerida enne uuringute käivitamist ühise koolituse ja androloogilise kliinilise läbivaatuse standardiseerimise teel.
2. Eesti-Leedu ja Eesti-Põhjamaade noorte meeste viljakuse uuringutes leidsime, et eesti meeste reproduktiivfunktsiooni markerite keskmised (mediaan) tulemused olid järgmised:
   2.1. munandi maht mõõdetuna Praderi orhidomeetriga mõlemas uuringus 22 ml (vasak munad) ja 23 ml (parem munand)
   2.2. spermatosoidide arv vastavalt 67 ja 57 milj/ml, normaalsete spermatosoidide protsent 7,7 ja 9,2 ning liikuvate spermatosoidide protsent 75 ja 74
   2.3. reproduktiivsete hormoonide Inhibiin B tase veres oli vastavalt 220 ja 198 pg/ml ning FSH tase veres oli vastavalt 3,4 ja 3,3 IU/l.
3. Noorte eesti meeste sperma kvaliteet ja teised reproduktiivse funktsiooni markerid on võrreldavad soome ja leedu meeste vastavate näitajatega ja paremad kui noortel meestel Taanis ja Norras, mis kinnitab ida-läänesuunalise meeste reproduktiivfunktsiooni gradiendi olemasolu Põhja- ja Baltimaade regioonis.
4. Spermatosoidide arvu ja Inhibiin B taseme vahel esineb positiivne korrelatsioon. Erandliku uuringugrupi – eesti sõdurite – tulemused, kus leidsime kõrgeima spermatosoidide kontsentratsiooni ja madalaima Inhibiin B taseme, viitavad, et selle hormooni regulatsiooni mehhanismid vajavad täiendavaid uuringuid.
5. Kliiniliselt tuvastatavad varikotseele ja munandimanuse patoloogia, läbipõetud munanditrauma, munandi- või munandimanuse põletik, mumpsorhiit, aga ka teostatud kubemesonga operatsioonide levimus on viljatute eesti meeste grupis statistiliselt kõrgem kui kontrollgrupis, mis viidab nende haiguste põhjuslikule seosele mehepoolse viljatusega Eestis.
6. Varikotseele on kõige levinum mehepoolse viljatuse riskitegur. Vasakpoolne varikotseele avaldab veenilaiendi raskusastmega seotud negatiivset mõju mõlema munandi mahule.
7. Kuna puuduvad absoluutsed kriteeriumid eristamaks viljatut ja viljakat meest, soovitame mehepoolset viljatust käsitlevates epidemioloogilistes

uuringutes grupeerida mehi vastavalt spermatosoidide arvule. Selline grupeerimine võimaldab täpsemalt hinnata erinevate patoloogiate ja riskitegurite suhtelist mõju mehe viljakusele.

8. Enamik uuringus tuvastatud olulistest mehe viljatuse riskiteguritest on ravitavad või isegi ennetatavad. Mehepoolse viljatuse riskitegurite senisest laialdasem teadvustamine nii elanikkonnale kui ka meditsiinisüsteemi erinevatel tasanditel loob edaspidiseks paremad eeldused nende vältimiseks, varaseks avastamiseks ja õigeaegseks raviks.

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PUBLICATIONS

I

Carlsen, E., Anderson, A.G., Buchreitz, L., Jorgensen, N., Magnus, O., V. Matulevicius, V., Nermoen, I., Petersen, J.H., Punab, M., Suominen, J., Zilaitiene, B., Giwercman, A. (2000) Inter-observer variation in the results of the clinical andrological examination including estimation of testicular size. Int J Androl, 23:248–253

II

Punab, M., Zilaitiene, B., Jorgensen, N., Horte, A., Matulevicius, V.,
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III

Jorgensen, N., Carlsen, E., Nermoen, I., Punab, M., Suominen, J., Andersen, A.G., Andersson, A.M., Haugen, T.B., Horte, A., Jensen, T.K., Magnus, O., Petersen, J.H., Vierula, M., Toppari, J., Skakkebaek N.E. (2002) East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. Hum Reprod, 17:2199–2208

IV

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Punab, M., Korrovits, P., Peetsalu A. (2003)
Meeste viljakust mõjutavad haigused. Eesti Arst, 82:181–187

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Teadustöö

Peamised uurimisvaldkonnad:

- meeste viljakus ja selle riskifaktorid
- meeste viljakuse geneetilised aspektid
- prostatiit ja leukotsütospermia
- meeste vananemine

Avaldanud 12 artiklit rahvusvahelistes (CC ja Medline tsiteeritud) ajakirjades, 5 artiklit ajakirjas Eesti Arst. Nelja raamatu, kahe ravijuhendi ja ühe filmi autor ja/või kaasautor.

Organisatsiooniline tegevus

- Euroopa Androloogia Akadeemia
- Balti Androloogide Selts – president
- Sugulisel Teel Levivate Infektsioonide Eesti Ühing – juhatuse liige
- Eesti Uroloogide Selts

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