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180

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Cytogenetic causes of male infertility

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CONTENTS

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Paper I: reception of the patients and controls, taking of blood samples, cytogenetic analyses of infertile males, data analysis, writing the paper.
Paper II: cytogenetic analyses of patient.
Paper III: reception of the patients, taking of blood samples, cytogenetic analyses of infertile males.
Paper IV: cytogenetic analyses, data analysis, writing the paper.
Paper V: reception of the patients, taking of blood samples, cytogenetic analyses of infertile males.
Paper VI: study design, reception of the patients and controls, taking of blood samples, cytogenetic analyses of infertile patients, data analysis, writing the paper.

ABBREVIATIONS

A	Arginine
AAT	Alpha-1 antitrypsin
AZF	Azoospermia factor
CECR	Cat eye syndrome critical region
CBG	C-bands by barium hydroxide using Giemsa
CFTR	Cystic fibrosis transmembrane conductance regulator
CNVs	Copy number variants
C	Cytosine
ESHRE	European Society of Human Reproduction and Embryology
FISH	Fluorescence in situ hybridization
FSH	Follicle stimulating hormone
G	Guanine
GTG	G-bands by trypsin using Giemsa
HH	Hypogonadotropic hypogonadism
ICSI	Intracytoplasmic sperm injection
IEF	Isoelectric focusing
Inv	Inversion
Inv dup	Inverted duplication
KS	Klinefelter's syndrome
LH	Luteinizing hormone
mar	Marker chromosome
mos	Mosaic
NOR	Nucleolus organising region
p	Short arm of chromosome
ps+	Increase in length of the satellite on the short arm of chromosome
pss	Double satellites on the short arm of chromosome
PRM1 and 2	Protamine genes 1 and 2
r	Ring chromosome
rob	Robertsonian translocation
q	Long arm of chromosome
qh-	Decrease in length of the heterochromatin on the long arm of chromosome
qh+	Increase in length of the heterochromatin on the long arm of chromosome
SCP	Single cell pathology
sSMC	Small supernumerary marker chromosome
SRY	Sex-determining region of Y chromosome
t	Translocation
T	Thymine
TNP2	Transition nuclear protein 2 gene
TP2	Transition protein 2
WHO	World Health Organization

INTRODUCTION

Reproductive health is a state of complete physical, mental and social well being in relation to the reproductive processes at all stages of life (WHO, 1996). Infertility is a relative condition with an involuntary reduction of the reproduction and increasing medical problem, touching male and female partners alike. In about a half of infertile couples, the male-related factor of infertility can be found. In about 1/3 of infertile couples a female factor is combined with a male disorder. The existence of some risk factors only in one partner is not necessarily associated with infertility of the couple, but may reduce the couple's fertility when present in both partners. Worldwide more than 70 million couples suffer from infertility. The number of infertile couples is growing about 1–1.5% a year, and the main causes of that is decreasing semen quality in males in the developed world, advanced maternal age, environmental factors, smoking or alcohol habits and stress. In developing countries the main factor of infertility is genital tract infections.

It is difficult to accurately assess the overall importance of the contribution of genetics to fertility, as most, if not all, conditions are likely to have a genetic component, for example susceptibility to infection. Nevertheless, a significant number of infertility phenotypes have been associated with specific genetic anomalies. The genetic basis of infertility is varying and includes chromosomal abnormalities, gene mutations and multifactorial causes. At present, with the advent of assisted reproductive technologies and the possibility of vertical iatrogenic transmission of genetic anomalies to the offspring, diagnosis of a genetic aetiology is very significant. Diagnosis is not only aid in determining the prognosis in these infertile patients but it also helps to counsel these couples about the birth of offspring with genetic anomalies. It is well known that a lower implantation rate and higher spontaneous abortion rate are closely related to the chromosomal abnormalities of both parents. An association between human infertility and chromosomal abnormalities has been known for long time. Infertile patients with a poor prognosis have an increased risk of having embryos with chromosomal abnormality, which could be one of the main reasons for implantation failure or recurrent spontaneous abortions. As a result, karyotyping of each person attending an infertility clinic would be necessary to identify those with genetic defects.

Understanding the basis of male infertility is an important part of providing complete care to an infertile couple. Fertility in the male may be lowered by an impairment of spermatogenesis or by the production of genetically unbalance gametes, which lead to loss of progeny through miscarriage. There are causes of male infertility that are treatable, either through medical or surgical management. Some conditions can be corrected to the point where the couple is able to conceive naturally or with assisted reproductive technologies.

In Estonia, data about chromosomal abnormalities in male infertility are still scarce. Previously, Käosaar (1973) has studied cytogenetically 28 infertile couple with recurrent spontaneous abortions. Polymorphic chromosomal

variants in normal adults were studied by Tüür (1974) and in Estonian female population by Kivi and Mikelsaar (1980).

Therefore, the main purposes of this research were to assess the presence and significance of cytogenetic causes of male infertility in Estonia. This study was carried out at the Chair of Human Biology and Genetics, Department of General and Molecular Pathology, Faculty of Medicine, University of Tartu, Tartu, Estonia.

REVIEW OF LITERATURE

1. Infertility of couples

Infertility is a failure to conceive after at least one year of unprotected intercourse (WHO, 2002). Infertility is an increasing medical problem affecting 10–15% of couples in their reproductive age. The number of infertile couples is growing and is related to the advanced age of the women who wish to become pregnant, and decreased semen quality in males in Western countries (Brugo-Olmedo et al., 2000; Swan et al., 2000; Punab et al., 2002; Practice Committee of the American Society for Reproductive Medicine, 2004; Tatone, 2008). The other causes are changes in lifestyle: increased number of sex partners with rise of incidence of sexually transmitted diseases; increased awareness of infertility and available treatments, resulting in a higher frequency of visits of infertile couples to the doctor (Brugo-Olmedo et al., 2000; Schmidt, 2006; Ochsendorf, 2008).

The male factor of infertility is detected in a half of infertile couples, and female factor in 22–35% of cases. In 14–24% of couples the cause of infertility remains unexplained (Pauer et al., 1997; Forti and Krausz, 1998; Huynh et al., 2002; Papanikolaou et al., 2005). Approximately 14–37% of couples who seek help for infertility usually exhibit a combination of factors from both partners. Couples with unexplained infertility have an approximately 40% probability of pregnancy within 5 years without treatment, and more than 30% of them will become pregnant within 3 years of appropriate treatment (Forti and Krausz, 1998). According to this, a spontaneous cumulative clinical conception rate after 12 months is approximately 20% for the infertile population (Gleicher et al., 1996). Among couples with a duration of infertility of 3 years or more, an additional year in the age of the female partner reduces the prognosis by 9% (Collins et al., 1989). The duration of infertility is one of the most significant prognostic indicators, as it shows the presence of genetic or other risk factors; the longer the duration of infertility is, the more powerful the underlying factors are (The ESHRE Capri Workshop Group, 2002). Couples with a history of primary infertility longer than 3 years are very unlikely to achieve an unassisted pregnancy (de Kretser, 1997; Gnoth et al., 2005).

The study of infertility must always be done considering the couple, both a man and a woman and interrelationship between them (Brugo-Olmedo, 2000). Both male and female-related causes of infertility may be genetic and non-genetic. The pathogenesis of male infertility can be reflected in defective spermatogenesis due to pituitary disorders, testicular cancer, germ cell aplasia, varicocele and environmental factors or in defective sperm transport due to congenital abnormalities or immunological and neurogenic factors. The causes of infertility in female partner include disorders of ovulation, endometriosis, tubal diseases, uterine and cervical factors, and immunological disorders (Brugo-Olmedo, 2000; Iammarrone et al., 2003; Jose-Miller et al., 2007).

Genetic causes have a considerable involvement in infertility. Moreover, with the use of *in vitro* fertilisation techniques, the genetic abnormalities may be transmitted to the offspring and hence create transgenerational infertility or other serious health problems. Therefore, genetic tests are a part of the diagnostic work-up of infertile individuals and should be performed before, during and after assisted reproduction including intracytoplasmic sperm injection (ICSI) (The ESHRE Capri Workshop Group, 2000; Gekas et al., 2001; Bonduelle et al., 2002; Foresta et al., 2002; Martin, 2008). Genetic causes, including chromosomal abnormalities and single gene defects could be present in approximately 11–30% of infertile males and 10% of infertile females (Pauer et al., 1997; Whitman-Elia et al., 2001; Foresta et al., 2002; Cruger et al., 2003; Rao et al., 2004; Vicdan et al., 2004).

Chromosomal alterations including chromosomal abnormalities and polymorphic variants have been found in 7.6–25% of infertile couples (Testart et al., 1996; Mau et al., 1997; Pauer et al., 1997; Meschede et al., 1998; Peschka et al., 1999; Wiland et al., 2001; Wiland et al., 2002; Morel et al., 2004; Mozdarani et al., 2008). Major chromosomal abnormalities are detected in 3.1–7.6% of infertile couples being higher in male partners (Pauer et al., 1997; Meschede et al., 1998; Scholtes et al., 1998; Gekas et al., 2001; Kayed et al., 2006; Mozdarani et al., 2008). Chromosomal polymorphic variants have been found in 8.7–58.7% of infertile male and 7.3–28.3% of infertile female partners versus 32.6% and 15.2% of fertile individuals, respectively (Mau et al., 1997; Wiland et al., 2002; Madon et al., 2005; Minocherhomji et al., 2009).

Numerical chromosomal abnormalities, predominantly trisomies of gonosomes and chromosomes 21, 18, 16 and 13, are the major cause of infertility and may be found in 21% of spontaneous abortions (Munné, 2002). The main factor that causes numerical chromosomal abnormalities is maternal age. Munné (2002) showed that the frequency of trisomies detected in amniocentesis was increased from 0.6% to 2.2% in females aged from 35 to 40 years (Munné, 2002). Most structural chromosomal abnormalities, like AZF deletions, are *de novo* events in parental germ cells. Others, e.g. reciprocal and Robertsonian translocations, as well as CFTR gene mutations, might be inherited (Mak and Jarvi, 1996). To find out causes of infertility in the individual couple, the genetic analyses are obligatory, especially when assisted reproduction techniques are used (The ESHRE Capri Workshop Group, 2002; Martin, 2008).

2. Male infertility and its causes

In half of infertile couples male-related causes are involved, being associated with impaired spermatogenesis and decreased sperm count (Yoshida et al., 1996; Tuerlings et al., 1997; Quilter et al., 2003). In 20% of couples, infertility is attributed to the male factor alone and from 30% to 50% of infertile couples a male factor is a contributory element. To be fertile, a man requires normal spermatogenesis, successful epididymal maturation, storage of sperm, normal

sperm transport and accessory gland function, along with appropriately timed intercourse. Normal spermatogenesis depends on the proper interaction of the hypothalamus, anterior pituitary gonadotropins and the testes. The most frequent diagnostic criterion for infertile men is sperm abnormality.

Factors contributing to male reproductive failure are often multiple and the relation between them is not always clear. In the diagnostic approach a detailed medical history, physical examination and sperm analysis are very important. The tendency of diminishing sperm quality is reported in Western countries including Estonia (Swan et al., 2000; Punab et al., 2002). Numerous non-genetic factors like age, chronic illnesses, infectious disorders, surgery, occupation, and lifestyle habits may influence male fertility and cause a decline of sperm quality. In cases of idiopathic decline of sperm quality a number of variable genetic factors may be found very frequently.

3. Genetic causes of male infertility: chromosomal abnormalities

Different genetic factors, including chromosomal abnormalities, single gene defects, and phenotypes with multifactorial inheritance (Table 1), have been considered in about 30–60% of infertile males (Tuerlings et al., 1997; Chiang et al., 2000; Gekas et al., 2001; Whitman-Elia et al., 2001; Quilter et al., 2003; Shah et al., 2003). Genetic abnormalities can lead to impaired reproductive function in adults, cause early foetal loss, genetic diseases or even death in offspring. Genetic defects may cause the dysfunction of the hypothalamo-hypophyseal-gonadal axis or interfere with the development of the male gonads and urogenital tract. They may cause arrest of germ cell production and maturation, or lead to the production of nonfunctional spermatozoa (Dada et al., 2006). Male infertility caused by a genetic defect will always be congenital (The ESHRE Capri Workshop, 2002).

Chromosomal alterations, including major chromosomal abnormalities and polymorphic chromosomal variants, have been found in 5.1–25% of infertile men, being 3.1–16.3% in males with oligozoospermia and up to 30% in men with azoospermia (van der Ven et al., 1997; Chiang et al., 2000; Dohle et al., 2002; Wiland et al., 2002; Cruger et al., 2003; Rao et al., 2004; Vicdan et al., 2004; Foresta et al., 2005; Dada et al., 2006).

Chromosomal abnormalities in infertile males have been found within the range of 2.4–16.4% (Pandiyan et al., 1996; Chiang et al., 2000; Gekas et al., 2001; Foresta et al., 2005; Elghezal et al., 2006; Ng et al., 2009; Koşar et al., 2010; Yatsenko et al., 2010) compared to the frequency in normal male population (0.3–0.4%) (Van Assche et al., 1996; Ravel et al., 2006). The most frequent are Robertsonian translocations and sex chromosome abnormalities. The impact of chromosomal abnormalities on male infertility is very high and inversely related to the sperm count.

Table 1. Classification of genetic causes of male infertility (Foresta et al., 2002)

Chromosomal abnormalities (homogenous or mosaicism)
Sex chromosomes
47,XXY (Klinefelter's syndrome)
47,XYY and other YY-aneuploidies
46,XX and 45,X males
Structural Y chromosomal abnormalities
Deletions
Rings
Isochromosomes
Inversions
Translocations
Autosomes
Translocations (Robertsonian, reciprocal)
Inversions
Other structural abnormalities (inversions, supernumerary marker chromosomes)
Clinical syndromes
Trisomy 21
Partial duplications and deletions
Chromosomal polymorphisms
Inv(9)
Familial inversion of the Y
Yqh+
Increased/reduced pericentromeric constitutive heterochromatin
Large-sized/duplicated satellites on acrocentric chromosomes
Gene mutations
Y-linked
Microdeletions Yq11
X-linked
Kalmann syndrome
Androgen insensitivity syndrome/Kennedy disease
Autosomal
Complex genetic syndromes in which infertility is a minor manifestation (myotonic dystrophy or 5-α-reductase deficiency)
Infertility as major manifestation
CFTR
Genes for β-subunit of LH and FSH and genes for LH and FSH receptors
Chromosomal alterations confined to sperms
Primary severe testiculopathies
Following radio-chemotherapy

In men with azoospermia the incidence of chromosomal abnormalities is especially high, varying from 13.1% to 23.6%. In males with oligozoospermia the incidence is 2.1–6.6% and in men with severe oligozoospermia it is 10.6%

(Yoshida et al., 1996; Bonaccorsi et al., 1997; van der Ven et al., 1997; Thielemans et al., 1998; Chiang et a., 2000; Dohle et al., 2002; Cruger et al., 2003; Rao et al., 2004; Vicdan et al., 2004; Dada et al., 2006; Elghezal et al., 2006; Ng et al., 2009). In infertile men with normal sperm count, the incidence of chromosomal abnormalities is about 3%.

Different hypotheses try to explain, how chromosomal abnormalities can alter spermatogenesis and cause reproduction failure in males but the exact mechanism is still not clear. As follows:

1) It might be related to the multitude of genes involved in spermatogenesis that could be deleted, truncated, or otherwise inactivated by the breakpoints of the chromosomal abnormality (Bugge et al., 2000). Breakpoints of the rearrangements may disrupt chromosomal regions where male fertility genes localise. Some of these genes may be dosage-sensitive or potential mutational targets for chromosomal breakpoints. A global interaction between the chromosomal rearrangement and the whole genome leading to impairment of sperm production is also suggested (Bugge et al., 2000; Ravel et al., 2006).

2) Synaptonemal disturbances may cause the impaired sperm production. The mechanical disruptions of the process of chromosome synapsis and chromosomal segregation during meiosis could possibly cause the germ cell destruction. In mice, asynapsed regions may trigger the meiotic checkpoint machinery to eliminate spermatocytes (Bache et al., 2004; Oliver-Bonet et al., 2005; Martin, 2008). A similar mechanism might explain why some chromosomal abnormalities in humans are associated with deficient spermatogenesis (Bache et al., 2004). In these cases, the location of the chromosomal breakpoint is important (Bugge et al., 2000).

3) It is reported that incomplete meiotic synapsis of rearranged chromosomes leads to centromeric association between the autosomal chromosome abnormality and the X-Y bivalent (sex body, X-Y body) (Oliver-Bonet et al., 2005). Transcriptional silencing of unsynapsed autosomal chromatin causes failure of proper transcriptional inactivation of the X chromosome at the mid-late-pachytene stage and therefore, overexpression of X-linked genes. It leads to a lethal gene dosage effect on the germ cells and to spermatogenic arrest (Johnson 1998; Homolka et al., 2007). This implicates that autosomal asynapsis in meiosis may cause male sterility due to meiotic failure and spermatogenic arrest in primary spermatocytes by interfering with meiotic sex chromosome inactivation (Homolka et al., 2007).

4) The meiotic arrest can also occur in the post-synaptic stage and is caused by inactivation of genes located on the regions associated with the X-Y body (Solary, 1999). This gene inactivation would block transcription of some other genes, which in turn trigger an apoptotic response. Unpairing of sex chromosomes followed by meiosis I arrest at the zygotene/pachytene stage cause apoptotic degeneration of germ cells and azoospermia (Vialard et al., 2006). As the result, cells surviving the first meiotic arrest would be eliminated later, resulting in the azoospermia in men (Oliver-Bonet et al., 2005).

Male meiosis suggested being more susceptible to the effect of chromosomal rearrangements compared to female meiosis (Bache et al., 2004). The association of chromosomal abnormality with X-Y body can explain the presence of meiotic arrest in males while females with the same chromosomal abnormality stay fertile (Johnson, 1998; Oliver-Bonet et al., 2005; Vialard et al., 2006).

3.1. Autosomal chromosome abnormalities

The incidence of autosomal chromosome abnormalities is 1.1–7.2% in infertile men, about 3% in the azoospermia group and up to 10.2% in the oligozoospermia group (Johnson, 1998; Chiang et al., 2000; Cruger et al., 2003; Vicdan et al., 2004; Foresta et al., 2005; Elghezal et al., 2006; Akgul et al., 2009; Yatsenko et al., 2010). Among normal fertile males autosomal abnormalities have been found in 0.2–0.3% (Van Assche et al., 1996; Ravel et al., 2006). Chromosomal abnormalities of autosomes can be structural or numerical.

Numerical autosomal abnormalities are a very rare cause of male infertility. Most numerical autosomal abnormalities are lethal. Male patients with trisomy 21 (Down's syndrome) are infertile.

Structural chromosomal rearrangements result from chromosome breakage with subsequent reunion in a different configuration. They can be balanced or unbalanced. In balanced abnormalities the chromosome complement is complete with no loss or gain of chromosomal material. The balanced rearrangements are usually harmless, but the carriers are often at high risk of spontaneous abortions or producing offspring with an unbalanced chromosomal complement. In case of unbalanced chromosomal abnormalities, the chromosomal complement contains an incorrect amount of chromosomal material. The clinical effects are usually very severe due to the imbalance of genes in genome, resulting in reproductive failure, mental retardation and physical problems.

Carriers of autosomal chromosome abnormalities are considered to be at risk of having oligozoospermia, recurrent spontaneous abortions or a child with a chromosomal abnormality (Yoshida et al., 1996). The following autosomal structural chromosomal abnormalities – translocations, supernumerary marker chromosomes and inversions – have been 7, 8, and 13 times, respectively, more frequently observed in infertile males compared to the normal newborn population (De Braekeleer and Dao, 1991; Krausz and Forti, 2000; Foresta et al., 2002; Huynh et al., 2002).

3.1.1. Translocations

Translocations are the most frequent structural abnormalities in infertile men (Bugge et al., 2000). Translocation (t) is a chromosomal abnormality caused by rearrangement of parts between non-homologous chromosomes. The resulted hybrid chromosome passes through meiosis forming trivalent or quadrivalent. About 2/3 of translocations arise *de novo,* but can also be familial (Bugge et al., 2000; The ESHRE Capri Workshop Group, 2002).

The importance of translocations relates to the pattern of their segregation in meiosis because the chromosomes involved in the translocation can't pair normally to form bivalents. The formation of the trivalent or quadrivalent may take more time than the normal process and defects in segregation during spermatogenesis might cause significant chromosomal imbalance leading to disturbances in spermatogenesis, early pregnancy loss or the birth of a child with multiple abnormalities. At pachytene stage of meiosis I the quadrivalent may associate with the X-Y body and interfere with X chromosome inactivation. The overexpression of X-linked genes leads to a lethal gene dosage effect on the germ cells and to spermatogenic arrest (Johnson 1998; Homolka et al., 2007). Other mechanism might be related to the multitude of genes involved in spermatogenesis. The linkage distance between two of them may be increased or new linkage relationships among these genes may arise because of an inserted translocated segment (Bugge et al., 2000). In newborns from the normal population the incidence of translocations was 0.2% (Stern et al., 1999; Mozdarani et al., 2008). Translocations may be reciprocal, non-reciprocal and Robertsonian.

Reciprocal translocations are a result of mutual exchange of any chromosomal segments between any two chromosomes. The resulting chromosome is called the derivative chromosome. The length of the exchanged segments may vary from a distal segment to the whole chromosome arms with breakpoints at the centromere. Reciprocal translocations have two or more breakpoints, or can be more complex rearrangements.

Balanced reciprocal translocations are usually inherited, have no any phenotypic effects but they may cause variable sperm conditions in carriers, ranging from normozoospermia to azoospermia (Van Assche et al., 1996). Even normozoospermic carriers are at risk of producing sperm with chromosomal imbalances leading to a reproductive failure (Morel et al., 2004). Depending on the chromosomes involved and the nature of the translocations, they can cause pre- and post-implantation losses, abnormal pregnancy outcome, or birth defects in the offspring (Shah et al., 2003). Incidence of reciprocal translocations in infertile men was 1.3–1.6%. In the oligozoospermic males the incidence was higher (1.3–1.7%) than in azoospermics (0–0.6%) (Pauer et al., 1997; Elghezal et al., 2006; Ng et al., 2009). In normal male newborns it was 0.2–0.3% (Yoshida et al., 1996; Bugge et al., 2000).

There are some suggestions how translocations can cause reproductive failure. In meiosis, reciprocally translocated chromosomes and their homologues align themselves in a cross shape known as a quadrivalent that can segregate during anaphase I in several different ways. At the pre-pachytene stage unpaired regions within the quadrivalent show a tendency to pair with each other when heterologous pairing is allowed by synaptic regulation that lead to first meiotic arrest. Extension of the X inactivation because of association between quadrivalent and the X-Y body leads to meiotic failure, spermatogenic arrest in primary spermatocytes and sterility in males (Oliver-Bonet et al., 2005; Vialard et al., 2006). The second meiotic arrest may be caused due to inactivation of genes located on the regions associated with the X-Y body and unpairing of sex chromosomes, causing an apoptotic degeneration of germ cells and azoospermia (Solary, 1999; Vialard et al., 2006). Synaptonemal disturbances or inactivation of involved in spermatogenesis specific genes may also cause the impaired sperm production in carriers of reciprocal translocations (Homolka et al., 2007). The type of chromosomes involved in the translocation, position of the breakpoints, size and characteristics of the translocated segments, presence or absence of recombination loci are responsible for spermatogenic breakdown (Bache et al., 2004).

About 16.7% of reciprocal translocations involve acrocentric chromosomes. These translocations are called non-Robertsonian and are more harmful for fertility of the carriers because of high tendency of acrocentrics to associate with the X-Y body causing severe spermatogenic defects (Guichaoua et al., 1990; Oliver-Bonet et al., 2005; Vialard et al., 2006). Among all chromosomes involved in rearrangements (including reciprocal translocations) in infertile males, the chromosome 1 is the most frequently reported. These rearrangements are *de novo* arisen or have maternal origin, but never paternal origin. So, they considered affecting male meiosis and fertility but not female fertility (Bache et al., 2004; Vialard et al., 2006). Possibly that breakpoints on chromosome 1 disrupt genes related to male germ cell development and integrity of large chromosomal domain of this chromosome is important for normal spermatogenesis (Bugge et al., 2000; Bache et al., 2004).

Non-reciprocal translocations are the result of a one-way transfer of a chromosomal segment from one chromosome to another.

Robertsonian translocations (rob) are the most common balanced chromosomal rearrangements in humans with an incidence of 1:1000 newborns. Robertsonian translocations originate from the centromeric fusion of the long arms of the acrocentric chromosomes (13–15, 21–22 and Y), usually with a simultaneous loss of both short arms. The fusion may be either heterologous or, rarely, homologous. The ISCN nomenclature describes rob as unbalanced whole-arm translocation (ISCN 2009). But due to the loss of short arms, which usually contain redundant DNA, the carriers, essentially, have a balanced chromosomal constitution with 45 chromosomes. Carriers of Robertsonian

translocation are usually phenotypically normal but often produce unbalanced gametes and have an increased risk for miscarriages and infertility. Compared with reciprocal translocations, Robertsonian translocations are 2.5 times more frequent in infertile males with more severe spermatogenesis impairment (sperm density lower than 10 mln/mL). Contrary, reciprocal translocations are predominant in men with sperm density over 10 mln/mL (Guichaoua et al., 1990). In infertile males Robertsonian translocations between the D (chromosomes 13–15) and G (21–22) group of acrocentric chromosomes are quite frequent, with prevalence of D/D translocations (up to 75%) (Thielemans et al., 1998). The incidence of Robertsonian translocations in infertile men is 2.0% (Stern et al., 1999; Elghezal et al., 2006). They have been found more frequently in males with oligozoospermia (1.6–3.4%) than in males with azoospermia (0.09–0.6%) (Johnson, 1998; Ogawa et al., 2000; Elghezal et al., 2006). The incidence in normal population is 0.08– 0.17% (Yoshida et al., 1996; Bonaccorsi et al., 1997; Johnson, 1998; Wiland et al., 2002). Most Robertsonian translocations (84%) are inherited.

The most frequent Robertsonian translocation is the t(13;14). Most cases are familial, and the same rearrangements have been found both in infertile and their fertile relatives. Different chromosomal breakpoints, with the occurrence of dicentric or monocentric Robertsonian translocations could explain phenotypic variability among carriers (Ravel et al., 2006). Rob t(13;14) is associated with variable phenotype from azoospermia to moderate oligozoospermia, and its incidence in infertile males is in a range of 1.5–2.3% (in normal newborns 0.07%) (Testart et al., 1996; Elghezal et al., 2006; Ravel et al., 2006). The incidence of rob t(14;21), associated with moderate oligozoospermia, is 0.4% in infertile men (Elghezal et al., 2006).

The reason for the occasional infertility in generations is still not known. During the first meiotic division chromosomes with Robertsonian translocation form trivalent that causes problems with pairing of homologs (Testart et al., 1996; Thielemans et al., 1998). The association between trivalent and X-Y body is frequent and explains severe spermatogenic defects. As a result, carriers of balanced translocations may produce three types of gametes: chromosomally normal, abnormal balanced and abnormal unbalanced. Preferential cis-configuration of meiotic trivalent structures supports alternate segregation (range: 60–93%) that results in production of normal and balanced gametes (segregation ratio is 1:1) and can lead to phenotypically normal offspring (Ogawa et al., 2000).

However, an adjacent segregation mode (range: 7–40%, with a mean 15%) results in unbalanced spermatozoa (Escudero et al., 2000; Martin, 2008), and abnormalities of other chromosomes, unrelated to the translocated chromosomes, may occur in 4.4% (Ogawa et al., 2000; Ogur et al., 2006). The abnormal unbalanced gamete can be disomic or nullisomic for one of the chromosomes with an extra or a missing chromosome q arm, respectively (Johnson, 1998; Scriven et al., 2001). As a consequence, unbalanced zygote with trisomy or monosomy for one chromosome appears. Zygotes with monosomy are not

compatible with life and most zygotes with trisomy result to pregnancy loss in the first trimester. Only some survive beyond the second trimester and to term (Scriven et al., 2001). If pregnancy evolves, there will be an increased risk for congenital abnormalities (Thielemans et al., 1998).

The actual risk of abnormal gamete production is difficult to quantify before fertilisation (Johnson, 1998; Scriven et al., 2001). The empirical reproductive risk for male carriers of translocation t(13;14) and t(14;21) was low with unbalanced gametes in a range of 10–26% and 8–18%, respectively (Rousseaux et al., 1995; Escudero et al., 2000; Frydman et al., 2001; Scriven et al., 2001). The risk of giving birth to a foetus with an unbalanced karyotype was 2.6% (Yoshida et al., 1997), empirical second trimester risks of chromosomally unbalanced liveborn with trisomy 13 (Patau syndrome) or trisomy 21 (Down's syndrome) were <0.5% and 3–5%, respectively.

3.1.2. Ring chromosome

The ring chromosome (r) is an aberrant circular chromosome resulting from deletions on both ends of the chromosome with a reunion of the proximal regions. Ring chromosomes have been found in all of the human chromosomes. The phenotype of carriers of the ring chromosome overlaps deletion syndromes for the same chromosome. The ring chromosome may affect spermatogenesis and cause male infertility because of meiotic arrest at the spermatocyte stage and instability (Johnson, 1998). The process of chromosome synapsis may be disturbed because of the structural effect of the abnormal (deleted) chromosome, and asynapsed regions may trigger the process of the germ cell destruction and elimination of spermatocytes (Bache et al., 2004). Incidence of ring chromosomes is 0.01% in the male newborn population, and 0.2% in infertile men with azoospermia (Yoshida et al., 1996).

3.1.3. Inversions

Inversion (inv) is a chromosomal rearrangement when a segment of a chromosome between two breakpoints is inverted 180 degrees and reintegrated into the same chromosome. Incidence of inversions in infertile men is 1–1.5%, in men with oligozoospermia 0.9% and azoospermia 0.1–0.3%. In the unselected newborn population it is 0.02–0.4% (Yoshida et al., 1996; Elghezal et al., 2006; Ravel et al., 2006; Riccaboni et al., 2008). The risk for congenital *de novo* inversions is 9.4% (Warburton, 1991). In normal fertile males the most frequent are inversions of chromosomes 2 and 10. Inversions of chromosomes 5, 6 and 11 are rare (Ravel et al., 2006).

Pericentric inversion – the inverted segment of the chromosome involves the centromeric region. It is the most frequent chromosomal rearrangement in

humans, with a frequency of 0.09–2.0% in the normal population (Ravel et al., 2006). Usually, balanced inversion does not have a phenotypic effect in the majority of heterozygotes. However, infertility, miscarriages and/or chromosomally unbalanced offspring can be observed in carriers of pericentric inversion (Mozdarani et al., 2008).

Pairing of homologous chromosomes is the main problem in inversion heterozygosis, which is more effective in males because of their high rate of meiotic division in the process of spermatogenesis (Mozdarani et al., 2008). In carriers of pericentric inversions the formation of a pairing loop can delay meiosis. Meiotic recombination is reduced within the pairing loop, leading to meiotic arrest (Elghezal et al., 2006). A number of crossover events during the pachytene stage between a normal and an inverted chromatid, or, in rare cases, also by U-loop recombination, results in production of unbalanced gametes. The crossover within the inversion segment can lead to two monocentric recombinants with reciprocal duplications/deficiencies in the sperm (Mozdarani et al., 2008). The frequency of unbalanced gametes varies from 0% to 54% (Martin, 2008). An overall risk for prenatal diagnosis has been estimated to be 10–15%, with 5% for males and 10% for females (Munné 2002). The genetic risk of inversion carriers depends on the size of inverted segments, and only inversions of length more than 100 Mbp would have a significant effect on fertility. In this case the incidence of unbalanced gametes is over 40%, related to the length of inversion. Only few unbalanced gametes are expected if the inverted segment is around 50 Mbp in length. No unbalanced gametes are expected in carriers of inversions with an inverted segment shorter than 50 Mbp (Anton et al., 2006). However, some authors reported pericentric inversions without any consequence on spermatogenesis; in particular, semen parameters were normal in all carriers (Ravel et al., 2006). In infertile males pericentric inversions of chromosomes 1, 3, and 9 have been most frequently reported (Bache et al., 2004) (see 3.3.).

Paracentric inversion – the inverted segment of the chromosome does not involve the centromeric region. These unbalanced chromosomes are produced by crossover. Paracentric inversion may occur in all chromosomes. The most common is inv(11)(q21q23). Inversions in 6p, 7q, and 14q are also reported. About 90% of paracentric inversions are inherited, and others are of *de novo* origin. Incidence of paracentric inversion in the infertile population is 2.0%, in spontaneous abortions 11.4%, and 0.1–0.5% in normal population (Pettenati et al., 1995; Vialard et al., 2007).

A large paracentric inversion (especially, of chromosomes 1, 3, 5, 6 and 10) could induce asynapsis at a pachytene stage resulting in silencing of some genes required for the progression of meiosis (Vialard et al., 2007). That causes impaired spermatogenesis and infertility (Krausz and Forti, 2000). About 4% of the offspring of paracentric inversions are a product of "U-loop recombination", which leads to either a duplication or deletion of part of the inverted segment (Pettenati et al., 1995). Chromosomally unbalanced offspring are usually lost

very early in pregnancy, perhaps during implantation (Vialard et al., 2007). The risk of aneuploidy for paracentric inversions has been estimated to be small (1–3.8%) (Vialard et al., 2007; Martin, 2008).

3.1.4. Small supernumerary marker chromosomes

Small supernumerary marker chromosomes (sSMCs) comprise a heterogeneous group of small structurally abnormal chromosomes that occur in addition to the normal 46 chromosomes. They are equal in size or smaller than chromosome 20 of the same metaphase spread, and cannot be identified definitely by conventional banding cytogenetics alone. sSMCs have been detected in connection with male fertility problems and found eight times more frequently among infertile males than in the normal population (De Braekeleer and Dao, 1991). The association between supernumerary marker chromosome and the X-Y body at the meiotic prophase causes meiotic arrest and instability (Guichaoua et al., 1990). That results in maturation arrest on the spermatocyte stage and impaired spermatogenesis (Chandley et al., 1987; Johnson 1998). The incidence of sSMCs carriers in infertile subjects is 0.125% with a male:female ratio of 7.5:1 (Liehr et al., 2007). In normal fertile men this frequency is 0.04% (Ravel et al., 2006). Approximately a half of the sSMCs are parentally transmitted (Manvelyan et al., 2008). sSMCs can lead both to fertility problems and repeated miscarriages (Liehr et al., 2007, Shah et al., 2003). The risk of congenital abnormality for *de novo* sSMCs depends on the involved chromosomes and their breakpoints, being between 11% (for satellited) and 14.7% (non-satellited SMCs) (Warburton, 1991)

Mosaicism is a condition when an individual has two or more genetically different cell lines that originate from the same zygote. Mosaicism with a normal cell line was found in 70% of cases with non-satellited markers and in 39% of cases with satellited markers (Warburton, 1991). About 60% of the sSMCs originate from chromosomes 14 or 15 (Manvelyan et al., 2008).

3.2. Sex chromosome abnormalities

Incidence of sex chromosome abnormalities in infertile men is in a range of 0.4–12.3%, being more frequent in azoospermia group (5.8–22.7%) than in oligozoospermia (0.5–4.4%) (Van Assche et al., 1996; Bonaccorsi et al., 1997; van der Ven et al., 1997; Johnson, 1998; Chiang et al., 2000; Dohle et al., 2002; Cruger et al., 2003; Rao et al., 2004; Vicdan et al., 2004; Foresta et al., 2005; Elghezal et al., 2006; Akgul et al., 2009; Yatsenko et al., 2010). In normozoospermic infertile men sex chromosome aneuploidies have been found in 1.4%, and in newborns in 0.1% (Johnson, 1998). The most frequent sex chromosome abnormality in infertile males is 47,XXY, Klinefelter's syndrome (Dada et al., 2006).

Numerical sex chromosome abnormalities include classic and mosaic forms of Klinefelter's syndrome, YY-aneuploidies and other numerical chromosomal abnormalities.

3.2.1. Klinefelter's syndrome

Klinefelter's syndrome (KS) is the most frequent sex chromosome disorder in infertile males, in which at least one extra X chromosome has been added to a normal male karyotype (Mak and Jarvi, 1996; Visootsak et al., 2006). The additional X chromosome(s) introduces lethal gene dosage in the testis environment that does not permit the survival of germ cells, resulting in azoospermia because of advanced germ cell atresia and aplasia (Johnson, 1998; Thielemans et al., 1998).

Incidence of KS is 3.1–7% in infertile males, 10.8–18.1% in males with azoospermia, and 0.4–1.3% in males with oligozoospemia (De Braekeleer and Dao, 1991; Tournaye et al., 1996; Yoshida et al., 1996; Johnson, 1998; Elghezal et al., 2006; Akgul et al., 2009; Yatsenko et al., 2010). The prevalence of KS is 1:660 in the general male population (Smyth and Bremner, 1998).

1) The classic form (karyotype 47,XXY) is found in 85–90% of cases. It results either from maternal (60%) or paternal (40%) meiotic nondisjunction of the X chromosomes (Yoshida et al., 1996). The incidence of XY disomy is considered to be increased in relation to age, indicating that older males and older females have an increased probability of producing 47,XXY offspring (Shah et al., 2003).

Many 47,XXY patients before puberty are normal but after puberty the concentration of testosterone has a tendency to decline and luteinising hormone to rise since defective Leydig cells will secrete insufficient amount of testosterone but high amount of estradiol. Because of the constantly elevated gonadotropins, the seminiferous tubules gradually become fibrotic and hyalinised. Their lumen will obliterate and their germ cells will gradually disappear, resulting in azoospermia and infertility. Hyperplasia of Leydig cells is generally found (Tournaye et al., 1996). The phenotype may vary from eunuchoid hypogonadism to normally virilised although sterile males, but the most constant features are small firm testes (95%) (testicular volume less than 10 mL), azoospermia and infertility, hypergonadotropic hypogonadism (50%), eunuchoid body proportions, large stature, degradation of secondary sex characteristics, gynaecomastia (in 12.5–88% of cases), and learning disabilities, sometimes a certain level of dyslexia and even mental retardation (Okada et al., 1999; Brugo-Olmedo, 2000). KS patients also frequently have other diseases of the lung, skin, liver and kidney (Morales et al., 1992; Swerdlow et al., 2005).

However, in 69% of KS patients have sperm in sperm extracts. Therefore, testicular sperm extraction and intracytoplasmic sperm injection may be considered in males with azoospermia and KS (Visootsak et al., 2006). Pregnancies with assisted fertilisation have already been reported (Brugo-Olmedo, 2000).

The incidence of variants 48,XXYY and 48,XXXY is in 1:17000–1:50000, and of 49,XXXXY in 1:85000–1:100000 male birth (Visootsak et al., 2006).

2) The mosaic form (karyotype 46,XY/47,XXY) (5–10% of cases) is a mitotic postzygotic event. A new cell line with karyotype 47,XXY appears at the stage of 4–5 cells (Mak and Jarvi, 1996; Tournaye et al., 1996). The more X chromosome present in the patient and the more abnormal cell lines in mosaic forms, the worse the testicular lesion and the more severe the manifestation of the syndrome (Mak and Jarvi, 1996; Brugo-Olmedo, 2000). Though Klinefelter mosaics can produce haploid sperm and normal children, they are at risk of meiotic abnormalities including nondisjunction (Johnson, 1998).

3.2.2. Other numerical sex chromosome abnormalities

Other numerical sex chromosome abnormalities were reported in a total of 1.8% of azoospermic men (Johnson, 1998).

1) 47,XYY and other YY-aneuploidies. Incidence of 47,XYY (YY-syndrome) in infertile males is in a range of 0.1–0.4% and is similar in groups with oligo- and azoospermia (Yoshida et al., 1996; Thielemans et al., 1998; Gekas et al., 2001). In normal adult fertile men the incidence is 0.05% (Ravel et al., 2006) and in male newborns 0.1% (Yoshida et al., 1996; Gekas et al., 2001; Shah et al., 2003). Extra Y chromosome in male karyotype appears *de novo* from nondisjunctions of the Y chromosome in paternal meiosis II. Most of 47,XYY males are phenotypically normal and potentially fertile. Sperm density varies from normal to azoospermia. The latter one is due to spermatogenic arrest of most of YY germ cells because of altered meiotic segregation, following sperm apoptosis and necrosis (Johnson, 1998; Moretti et al., 2007). Testicular biopsies show often maturation arrest to complete germinal aplasia (Mak and Jarvi, 1996). The risk of having abnormal children is not higher compared to the normal 46,XY male, since the extra Y chromosome is eliminated early during spermatogenesis and does not pass into the next generation (Mak and Jarvi, 1996). The mosaic form 47,XYY/46,XY found in 0.27% in infertile males, and other types, such as 47,XYY/49,XYYYY/46,XY and 45,X/47,XYY in 0.09%. In these cases, a mitotic nondisjunction occurs in the early embryonic stage.

2) 46,XX sex reversal males. Incidence of 46,XX males is 1/20 000 in male newborns (Mak and Jarvi, 1996), and 0.9% in men with azoosperma. Sterility is caused by absence of the azoospermia factor (AZF) on the long arm of the Y chromosome, which is necessary for the initiation and maintenance of spermatogenesis (Yoshida et al., 1996). 46,XX males have small, firm testes and azoospermia. Testicular biopsies show either Sertoli or Leydig cells, and hyalinisation and fibrosis of seminiferous tubular (Yoshida et al., 1996).

3) 45,XO/46,XY mosaic males or mixed gonadal dysgenesis. Incidence in male newborns is 0.07/1000, in oligozoospermics 0.2% and in azoospermics 0.1% (Yoshida et al., 1996). Approximately in 33% of cases patients are raised

as males. Most of them are sterile, with azoospermia in 2/3 of patients and oligozoospermia in the remains (Yoshida et al., 1996). One-third of patients display features of Turner syndrome (Thielemans et al., 1998).

Structural sex chromosome abnormalities include sex chromosome translocations and structural Y chromosomal abnormalities, from which the most frequent are Y chromosome deletions, especially, microdeletions of the AZF region.

3.2.3. Sex chromosome translocations

Usually translocations occur between Y chromosome and an autosome, X-Y translocations are rare. Sterility in these males is due to interference with inactivation of the X-Y bivalent and spermatogenesis impairment (Yoshida et al., 1996). The incidence of translocations between a gonosome and an autosome is 3:1000 in males with azoospermia and 0.45:1000 in male newborns (Yoshida et al., 1996).

X-autosome translocations. Reciprocal X-autosome translocations always cause male infertility because of severe spermatogenic arrest and azoospermia. X-linked loci from translocated segment of X chromosome become linked to elements controlling autosomal gene expression. The abnormal X-inactivation of translocated segment influences the genetic control of germ cell progression, resulting in meiotic arrest at the primary spermatocyte stage (Johnson, 1998).

Y-autosome translocations. Reciprocal translocations of Y chromosome may impair spermatogenesis due to abnormal sex chromosome pairing during meiosis, abnormal testis determination in the indifferent gonad (impaired SRY) or defective transcription of the AZF factors (Johnson, 1998).

Dicentric Y chromosome. Incidence of a dicentric Y chromosome is 0.3% among infertile men with azoospermia (Yoshida et al., 1996). Probably, a dicentric Y chromosome is arisen via tandem translocation between two Y chromosomes attached end-to-end (Herva et al., 1980). One of the centromeres forms the primary (functional) constriction; the other one is presumably inactive and detectable only as C-positive material on each chromatid. The presence of two centromeres disturbs pairing between X and Y chromosomes and their normal segregation in meiosis and cause the delay in anaphase (Yoshida et al., 1997). Influence on phenotype depends on the part of the Y chromosome, which is missing (Yoshida et al., 1996). The phenotype varies from Turner-like females to normal males. Most of them are azoospermic with maturation arrest at the primary spermatocyte stage in testicular biopsies.

3.2.4. Structural Y chromosome abnormalities

Different structural Y chromosome rearrangements may affect fertility due to defects of genes, involved in spermatogenesis. The most frequent are Y chromosome deletions, inversions, rings, isochromosomes and translocations.

Isochromosomes – i(Y). A metacentric chromosome with equal length and genetically identical arms, is produced during mitosis or meiosis by transverse splitting of the centromere of Y chromosome. The effect on spermatogenesis depends on either short or long arm of Y chromosome are involved in i(Y). Isochromosome Y has been reported in association with severe spermatogenic defects (Lin et al., 2005), but its incidence is very rare.

Inversions – inv(Y). The incidence of pericentric inv(Y) chromosome is 0.1% in infertile men and 0.037% in male newborns (Yoshida et al., 1996). Pericentric inv(Y) has been reported in the association with microdeletion of Y chromosome in infertile males with severely depressed spermatogenesis (Iwamoto et al., 1995; Causio et al., 2000; Tomomasa et al., 2000).

Deletions. The frequency of Y chromosome deletions increases with the severity of spermatogenic defect. The incidence in infertile men is 5–20% (Yoshida et al., 1996; Foresta et al. 2002) whereas 5–10% in oligozoospermic males (Dada et al. 2006) and 15–20% in men with idiopathic non-obstructive azoospermia or severe oligozoospermia (Foresta et al. 2002).

1) Microscopically visible Y chromosomal deletions are one of the common causes of severe spermatogenic defects. Structural aberration in Y chromosome involving SRY/AZF genes may result in a variety of clinical states with decreased fertility (Thielemans et al., 1998).

2) Microdeletions in the AZF region. Microdeletions of the long arm of the Y chromosome (Yq) have been found in 5.5% of infertile men, in 10–13% of men with non-obstructive azoospermia or severe oligozoospermia (range from 1% to 55% in different studies) (Dada et al., 2006; Ng et al., 2009) and 0.01–0.02% in male newborns (Mak and Jarvi, 1996; Le Bourhis et al., 2000). These deletions are usually of *de novo* origin and are rarely transmitted to offspring. Three different spermatogenesis loci (a, b and c) have been assigned to the azoospermia factor region (AZF region) on Yq11, where different candidate genes have been mapped: USPY9 and DBY in AZFa, RBMY1 in AZFb and DAZ in AZFc (Foresta et al., 2002; O'Flynn O'Brien et al., 2010). AZF microdeletions may cause 1) deregulation of gene expression by position effect; 2) interfere with post-transcriptional modification of gene expression; or 3) may also result in the absence of several genes critical for spermatogenesis causing spermatogenic failure (McElreavey et al., 2008).

Rings – r(Y). An aberrant circular derivate of the Y chromosome occurred as a result of deletions at both telomeres of the Y chromosome and reunion of proximal regions. Deleted small distal fragments have been removed during cell divisions. Most patients have a mosaic 46,X,r(Y)/45,X karyotype. The phenotype is depending on the percentage of the monosomic cell line in the different tissues and on the regions deleted during the ring Y formation (Arnedo

et al., 2005). An r(Y) with low material loss can be naturally transmitted, showing similar mitotic behaviour in the offspring. The presence of the r(Y) chromosome in germinal cells increased the risk of fathering offspring with numerical abnormalities, even for chromosomes not involved in the arrangement (Arnedo et al., 2005).

3.3. Polymorphic chromosomal variants

Chromosomal alterations include minor or "normal" polymorphic chromosomal variants or polymorphisms of heterochromatic regions in addition to major chromosomal abnormalities. Polymorphism means the presence of several or many morphological variants in the population with a frequency of 1% or more (Neel, 1978). The term "variant" was recommended at the Paris Conference (1971) and means the deviations from the norm of chromosome morphology. The term "heteromorphic" has been recommended to describe the homologous chromosomes with arms of different length or variable bands (Bhasin, 2005). In the narrow sense it means differences between homologous chromosomes, and in the broad sense the term heteromorphism is used synonymously with polymorphism or normal variant (Sahin et al., 2008).

Heterochromatin is of two types, facultative and constitutive. Facultative heterochromatin is a reversible form, not rich in satellite DNA and not very polymorphic and becomes transcriptionally active in some periods of the cell cycle (Yakin et al., 2005). It is found in the inactive X chromosome or Barr body. Constitutive heterochromatin is formed by tandemly organised short nucleotide sequences of satellite DNAs that do not encode proteins, and is very polymorphic and unstable. It contains two types of satellite DNA. "Classical" satellite I, II, III or IV DNAs and alpha-satellite (alphoid) DNA. Constitutive heterochromatin remains transcriptionally inert during the entire cell cycle (Yakin et al., 2005). Variations have been caused by the different amounts of tandemly repeated DNA sequences: satellites I, II, III and IV, rRNA genes, and β satellite, which are located on the short arms of acrocentric chromosomes in p11, p12 and p13 bands, respectively. A larger amount of heterochromatin is usually associated with the specific chromosomal regions (Bhasin, 2005), which could be visualised with conventional staining procedures, such as C- or Q-banding techniques:

1. Variants of heterochromatic regions of chromosomes 1, 9 and 16: elongation of long-arm (q) region (qh+) or contraction (qh-) due to partial duplication or deletion of the heterochromatic segment itself, and partial or full pericentric inversion of the heterochromatic region.

2. Variation in the length of the long arm of the Y chromosome due to duplication (qh+) or deletion (qh-) in the large heterochromatic segment. The length of the human Y chromosome varies in different population groups and from man to man.

3. Polymorphic variants of short-arm (p) of acrocentric chromosomes in the D (13–15) and G (21–22, Y) groups: enlargement (ph+) or absence (ph-) of the short arm of acrocentrics; enlargement (ps+) of satellites, tandem satellites (pss+), some very rare variants like streak or multiple satellites or split satellites. On acrocentric chromosomes, the nucleolus organising region (NOR) on the stalks (pstk) of satellites consists of rRNA, while the short arm (p) and satellites (ps) consists of heterochromatin.

Variations of the heterochromatic material can also be seen in other chromosomes. Some rare variants of chromosome 4 and 18qh+ were previously reported without any risk of phenotypic abnormalities (Kowalczyk et al., 2007).

Polymorphisms of heterochromatic regions are individually stable and frequent in the normal population. Most polymorphic variants are familial and follow Mendelian inheritance from one generation to another with a low mutation rate (Bhasin, 2005). *De novo* polymorphic chromosomal variants are rarer and appear, possibly, as a result of an unequal crossover between heterochromatic regions of homologous chromosomes in meiosis. It is possible due to conjugation of repeated DNA sequences. *De novo* heterochromatic variants are considered to be larger in size and to be associated with clinical conditions. Because of the high variability of the variants only monozygotic twins have the same heterochromatic variants (the concordance is approximately 100%), whereas in dizygotic twins the concordance rate is about 50%. Variations in the incidence of polymorphic chromosomal variants are mainly due to ethnic or racial differences. Smaller C-band-sizes of chromosomes 1, 9 and 16 have been reported in the Japanese population and in blacks when compared to Caucasians. A north/south gradient in the length of Y chromosome in Europeans is also suggested with the longest Y chromosome in men of the Mediterranean origin. In addition, variations in different tissues have been reported with significantly higher frequencies of polymorphisms in amniocytes than in lymphocytes (Bhasin, 2005).

The function of satellite DNA and heterochromatin in cells is still unclear. It is suggested that heterochromatin may have several functions, such as structural, protective, metabolic, evolutional and transcriptional functions (Prokofjeva-Belgovskaja, 1977). It is suggested that constitutive heterochromatin not contain structural genes, and is probably associated with control and functioning of the organisation of the nucleolus and production of ribosomal DNA, stabilisation of chromosome structure, recognition and attraction homologues at meiosis. Possibly, it provides raw material for new genes, acts as a gene spacer and loci for recombination and serves as an "absorbent" for mutagens, carcinogens, clastogens, etc. (Bhasin, 2005). Satellite DNA may also have a role in the process of X chromosome inactivation (Warburton et al., 2008).

Hennig (1999) proposed that molecular composition of the chromatin in heterochromatic regions is generally similar to that in silenced chromosomal regions. The similar epigenetic signals in DNA (such as gene promoter hypermethylation and hypoacetylation of histone tails) initiate chromatin packaging of

heterochromatin and are necessary for the inactivation or silencing of genes. It is reported that heterochromatic regions of specific chromosomes (for example 9q11-q12) may modify expression of other genes through transcriptional activation, which arise in response to heat shock and is similar to that of active euchromatic regions (Minocherhomji et al., 2009). Stress-induced activation of heat shock protein genes is controlled by the heat shock transcription factor 1(HSF1), which forms nuclear stress granules on the 9q12 locus. Because of binding of HSF1 to satellite III repeated elements, the RNA polymerase II-dependent transcription of these sequences drives into stable RNAs, which remain associated with 9q12 region for a certain time after synthesis, even throughout mitosis. Also upon stress, targeting and retention of other proteins into the granules on satellite III transcripts have been reported (Madon et al., 2005).

The function and the effects of polymorphic variants on phenotype are still poorly understood, and the literature data are still controversial. Because of highly complex and heterogeneous heterochromatin, extensive variations of polymorphic variants are possible without any harmful effect on phenotype and are because of that considered to be a normal variant (Hamer et al., 1981; Foresta et al., 2002; Bhasin, 2005; Kowalczyk et al., 2007). The others reported the increased frequency of variants in association with different clinical conditions such as reproductive failure, recurrent spontaneous abortions and even psychiatric disorders (Madon et al., 2005; Minocherhomji et al., 2009). In infertile men the incidence of polymorphic chromosomal variants have been reported within the very large range from 4.9 to 58.7% (Nielsen, 1988; Nakamura et al., 2001; Penna-Videau et al., 2001; Wiland et al., 2002; Madon et al., 2005; Nagvenkar et al., 2005; Minocherhomji et al., 2009) as opposed to 10–15% in the normal population (Hamer et al., 1981).

The association between chromosomal polymorphisms and impaired spermatogenesis is still not clear. It is suggested that polymorphic variants have an adverse effect on male reproductive function associated with abnormalities in the hypothalamic-pituitary-testicular axis that could predispose to aberrant gametogenesis and subsequent foetal wastage (De Braekeleer and Dao, 1991; Sasagawa et al., 1998). Possibly, large heterochromatic blocks are responsible for the weakening of chromosome pairing, spindle fibre attachment, or down-regulation of normally expressed or active genes, leading to meiotic arrest and infertility in men. It is also suggested that there may be the association of heterochromatic blocks with the silencing of gene expression, particularly genes associated with spermatogenesis and other fertility/infertility-associated genes (Minocherhomji et al., 2009). Changes in the structural element of the centromere due to polymorphic heterochromatin may lead to defective chromosome segregation during spermiogenesis, and an increased rate of sperm aneuploidy (Yakin et al., 2005). These males have a lower expectancy for a positive outcome in assisted reproduction technologies (Yakin et al., 2005).

Variants of chromosome 1

In infertile males the incidence of 1qh+ has been reported in a range from 0.5% to 3.6% (0–8.1% in normal population), but variant 1qh- and inv(1)(p11q12) have not been described. Large inversion, inv(1) (p13q21) is a rare variant in the normal population, observed only among normal Estonian adults (0.2%) and in Indian newborn males (Tüür et al., 1974; Nielsen, 1988; Nakamura et al., 2001; Penna-Videau et al., 2001; Wiland et al., 2002; Bhasin, 2005; Nagvenkar et al., 2005).

The polymorphisms of 1qh have been reported in the relationship with foetal wastage, recurrent miscarriage or malignant diseases by some authors. In inversion, inverted segment may cause synapsis failure, including asynapsis or early desynapsis, and pairing abnormalities of homologs leading to male infertility (Bhasin, 2005). In general, inversions of heterochromatic regions are considered not to cause phenotypic abnormalities.

Variants of chromosome 9

It is the most frequent heterochromatic variant both in the normal population and infertile males. The heterochromatic variant 9qh+ has been observed in 0.3–14.3% of infertile males (Nakamura et al., 2001; Penna-Videau et al., 2001; Wiland et al., 2002) and in up to 12.9% of the normal population (Bhasin, 2005). The incidence of 9qh- variant is up to 24.4% in the normal population (Bhasin, 2005), and 0.5–5% in infertile males (Nielsen, 1988; Wiland et al., 2002). In some reports the variant 9qh+ has been found in the association with repeated spontaneous abortions and malformed stillborn infants. It has been found more frequently (8%) in children with *de novo* major chromosomal abnormalities than in normal newborns (0.04%) (Bhasin, 2005). It is suggested that 9qh+ play significant roles in chromosomal nondisjunction. Large heterochromatic blocks may cause chromosome impairment and meiotic arrest resulting in infertility. Nevertheless, other studies have not found significant differences in polymorphic variants of chromosome 9 between patients and controls (Bhasin, 2005).

Pericentric inversion 9, especially complete inv(9)(p11q13) has been reported in association with reproductive failure (Table 2).

Incidence of inv(9)(p11q13) is 1–2% in the normal population, whereas up to 5% in infertile males (Nakamura et al., 2001; Nielsen, 1988; Penna-Videau et al., 2001; Wiland et al., 2002; Collodel et al., 2006; Mozdarani et al., 2007).

Inversion 9 has been considered to play significant role in chromosomal nondisjunction, and have variable effects on spermatogenesis, from azoospermia to severely altered sperm morphology, motility and meiotic segregation (Collodel et al., 2006). In chromosomes with inversion, a loop will be formed during meiosis I that can lead to production of abnormal and unbalanced gametes. Carriers of such inversion are at risk of having an offspring with unbalanced karyotype. It is suggested that inv(9) might have also some interchromosomal effect leading to a higher incidence of mitotic disturbances and it is known to be associated with aneuploidies such as mosaic trisomy 21 (Madon et al., 2005).

Table 2. Associations between the infertility and inv(9) (p11q12) or inv(9)(p11q13)

Karyotype	Disturbance	Reference
Inv(9)(p11q12)	Infertility/recurrent miscarriage	Sasiadek et al., 1997
Inv(9)	Male infertility	Sasagawa et al., 1998
Inv(9)	Recurrent spontaneous first trimester miscarriage	Parmar and Sira, 2003
Inv(9)(p11q12)	Infertility/recurrent miscarriage	Mozdarani et al., 2007
Inv(9)	Immotile/ultrastructural sperm defects	Baccetti et al., 1997
Inv(9)(p24q13)	Male sterility	Davalos et al., 2000
Inv(9)	Male infertility	Collodel et al., 2006
Inv(9)(p11q13)	Infertility/recurrent miscarriage	Srebniak et al., 2004
Inv(9)	Infertility	Düzcan et al., 2003
Inv(9)(p11q13)	Subfertility	Teo et al., 1995
Inv(9)	Sertoli-cell- only tubule	Tomaru et al., 1994
Inv(9)	Intrauterine foetal death/infertility	Uehara et al., 1992
Inv(9)	Depression of spermatogenesis/infertility	Faed et al., 1982

Variants of chromosome 16

In infertile males the incidence of variant 16qh+ varies from 0.9% to 1.9% (0–6% in normal population) (Nielsen, 1988; Nakamura et al., 2001; Bhasin, 2005; Nagvenkar et al., 2005; Yakin et al., 2005). Although, the incidence of 16qh- and the pericentric inversion, inv(16)(p11q11) varied in large range (0.04%-23.6% and 1.4%, respectively), they have not been found in infertile men (Hamer et al., 1981; Bhasin, 2005).

Variants of the Y chromosome

The Y chromosome shows a wide range of variation not only between individuals but also between different population groups. The incidence of Yqh+ is rare in newborns from England (0.14%) but frequent in Australian whites (40%) (Bhasin, 2005). In normal Estonian males its incidence is 4% (Mikelsaar et al., 1975).

The data about clinical significance including fertility of polymorphisms of the Y chromosome are still controversial. Variant Yqh+ has been reported in association with reproductive failure. Possibly, it has been caused by the inhibition of gene transcription due to the silencing effect on the genes promoters in close proximity (Madon et al., 2005). In infertile males the incidence of Yqh+ is 4.5–7.9% (Penna-Videau et al., 2001; Madon et al., 2005; Nagvenkar et al., 2005). Minocherhomji et al. (2008) found Yqh+ variant even in 26.9% (102 of 380) of infertile males.

The short Y chromosome (Yqh-) varies from 0.11% in Canadian military cadets (Bhasin, 2005) to 7% in normal Estonian males (Mikelsaar et al., 1975). The data of the incidence of Yqh- in infertile males are conflicting. Some authors reported its incidence 0.5–1.3% (Nielsen, 1988; Minocherhomji et al., 2009) and even 27.2% (Nagvenkar et al., 2005), but other authors have not found any association between an increased risk of pregnancy loss and Yqh- variant in carriers.

4. Genetic causes of male infertility: gene defects

The systematic chromosomal study is a powerful approach not only to find chromosomal causes of male infertility, but also through revealed chromosomal abnormalities to detect regions where fertility genes could be localised. Over 2000 genes act at different stages of germ cell development and thought to be involved in the control of human male development and reproduction (Fritsche et al., 1998; Ramanujam et al., 2000; van der Ven et al., 2000; Bezold et al., 2001*;* Huynh et al., 2002; Reynolds et al., 2005):

1) Genes involved in spermatogenesis. Mutations in these genes cause meiotic arrest resulting in azoo- or oligozoospermia. The mostly studied genes from them: CREM (10p11), UBE2B (5q31), RBM (Y p and q), AACT (14q32), OAZ3 (1q21), BAX (19q13), DHH (12q13), Ptch1 (9q22), SPO11 (20q13), SYCP3 (12q), DAZL (3p24), MLH1 (3p21), DMC1 (22q13), MSH4 (1p31), CYP1A1 (15q22-q24), numerous genes from long arm of Y chromosome Yq11: USP9Y, DBY, UTY, EIF1AY, RBMY, DAZ, CDY1, BPY2, PRY, TTY2 etc.

2) Genes involved in development and differentiation: SRY (Yp13), SOX9 (17q23), CFTR (7q31), BMP4 (14q22-q23), INSL3 (19p13-p12), LGR8 (13q13), cKIT (4q11-q12), BMP8 (1p35-p32), DMRT1 (9p24), SF1 (11q13). Mutations in these genes cause abnormalities in sex differentiation, development of male reproductive system, affect migration and proliferation of primordial germ cells.

3) Genes involved in function of endocrine system: Fkbp6 (7q11), GNRH1 (8p21-p11), GNRH2 (20p13), AR (Xq12), LHß (19q13), LHCGR (2p21), KAL1 (Xp22), FSHß (11p13), FSHR (2p21-p16), HLA (17), GATA4 (8p23-p22). Mutations in these genes affect sex differentiation and cause sperm pathology.

4) Genes involved in sperm functions: PRM1 (16p13.3), PRM2 (16p13.3), TNP1 (2q35-q36), TNP2 (16p13), CSNK2A2 (16q21), APOB (2p24-p23), ROS1 (6q22), ADAM2,3 (8p11), CATSPER2 (15q15), AKAP82 (Xp11.2). Mutations in these genes cause decline of sperm quality, affect sperm penetration, acrosome reaction and pronuclear reaction.

Some genes act on their own, some acting in collaboration with other genes and also interacting with external factors (The ESHRE Capri Workshop Group, 1998). The genes related to male infertility locate on different chromosomes involving mostly gonosomes, predominantly Y chromosome. Especially the long arm of the Y chromosome contains numerous genes (AZF, etc.) and gene families considered candidates for azoospermia (see 3.2.4.). Large deletions are generally associated with more severe spermatogenic defects. Microdeletions of Yq11 region are found almost exclusively in males with azoospermia or severe oligozoospermia. Microdeletions of this region has been termed AZFa (including DFFRY, USP9Y, DBY, UTY genes), AZFb (including EIF1AY, RBMY genes), AZFc (including DAZ, CDY1, BPY2, PRY, TTY2 genes) and AZFd (Huynh et al., 2002). Relatively uncommon AZFa deletions are generally associated with Sertoli-cell-only syndrome, while most common AZFb and AZFc deletions may be associated with a variety of defects (Shah et al., 2003).

Mutations of genes on X chromosome cause infertility in patients mainly with Kallmann syndrome and Kennedy disease (The ESHRE Capri Workshop Group, 2002; O'Flynn O'Brien et al., 2010).

Infertility may be the only clinical manifestation of a gene defect in an otherwise phenotypically normal individual, but it can also be one of the symptoms of monogenic disease.

AIMS OF THE STUDY

The general aim of the study was to assess the role of chromosomal abnormalities in male infertility.

The study had the following tasks:

1) To investigate the presence of chromosomal alterations in male patients with infertility, and controls.
   a) To assess the impact of major chromosomal abnormalities in male infertility.
   b) To assess the impact of heterochromatic polymorphic chromosomal variants in male infertility.
2) To study the origin of detected chromosomal abnormalities and discuss their role in male infertility.
3) To analyse in infertile males the specific role of genes in detected chromosomal abnormalities: to confirm the presence of known genes and propose the existence of novel regions for male fertility.
4) To investigate other genetic factors (alpha-1 antitrypsin), which influence on the phenotype of the infertile patients with chromosomal abnormalities.

MATERIAL AND METHODS

5. Patients and study design

The study was carried out at the Chair of Human Biology and Genetics, Department of General and Molecular Pathology of the University of Tartu. Written informed consent, confirmed by the Ethics Review Committee on Human Research of the University of Tartu, was obtained from each participant. Chromosomal studies were performed in 90 infertile men with disturbances of spermatogenesis. As a control, chromosomal analyses in 30 healthy fertile men were performed (Table 3).

Table 3. Characteristics of study groups

Study group (n)	Period of study	Description	Paper	Mean age ± SD years
Infertile male (n=90)	1999–2004	Infertile men with azoo- and/or oligozoospermia	I, II, III, IV, V, VI	31.8 ± 5.3
Infertile males with Klinefelter's syndrome (n=13)	1999–2008	Infertile men with azoo- and/or oligozoospermia	III	35.5± 11.2
Control (n=30)	2003–2004	Fertile males-volunteers	I, VI	41.4 ± 9.5

Infertile male group. A total, 90 infertile males with mean age±SD 31.8±5.3 years underwent the study from December 1999 to November 2004. They were referred to Institute of General and Molecular Pathology, Department of Human Biology and Genetics by andrologists or urologists from Estonia. Before referral to the chromosomal analyses, patients underwent thorough physical examination, hormonal tests and at least two semen sampling at the Andrology Unit of the Tartu University Hospital. Semen samples were assessed according to the criteria given by WHO (WHO 1999) (Table 4) with slight modifications by Andersen and co-workers (Andersen et al., 2000).

According to sperm density the patients were classified into groups:

1) Patients (n= 32) with azoospermia (no spermatozoa in seminal fluid)
2) Patients (n= 58) with oligozoospermia (less then 20 mln spermatozoa per mL), from which
   – patients (n=53) with severe oligozoospermia (0.1–5 mln spermatozoa per mL)

– patients (n=5) with mild and moderate oligozoospermia (5–20 mln spermatozoa per mL)

Infertile male group with Klinefelter's syndrome. To explain the association between the KS and AAT deficiency 13 infertile males with KS were studied. From these 13 males 5 patients were from above mentioned infertile male group (n=90). Other 8 patients were added later from the subsequently studied infertile men, sent by andrologist for the chromosomal analysis from December 2004 to December 2008. They mean age± SD was 35.5 ± 11.2 years.

Control group. 30 healthy fertile men-volunteers, who had at least two children, were selected as a control group. They mean age± SD was 41.4 ± 9.5 years.

Table 4. Normal sperm parameters (WHO, 1999)

Parameters	Normal references
Volume	≥2 mL
pH	≥7.2
Sperm density	≥20 mln/mL
Total sperm count	≥ 40 mln/mL per ejaculate
Motility	≥ 50% with motile (grades A+B) or ≥ 25% with progressive motility (grade A) within 60 min of ejaculation
Morphology	> 15% with normal forms
Vitality	≥ 50% live
White blood cells	< 1 mln/mL
Immunobead test	< 50% motile spermatozoa with beads bound
Mixed agglutination reaction test	< 50% motile spermatozoa with adherent particles

6. Methods

6.1. Chromosomal analyses

Chromosomal analysis was carried out from the peripheral blood lymphocyte cultures. The karyotype of each patient was determined by GTG method (G-banding method using trypsin and Giemsa stain). Studies of chromosomal polymorphisms were performed by CBG-method (C-banding method using barium and Giemsa). Other methods, such as R-, Q-, AgNOR and fluorescence in situ hybridization (FISH) methods were used for the identification of chromosomal abnormalities. In each patient 35 metaphases were analysed. In cases of mosaicism (see at the end of 3.1.4.) 50–100 cells were examined. Chromosomal abnormalities present in more than two cells were considered as

mosaicism (ISCN, 2009). Chromosomal abnormality confined to a single cell was considered as an artefact, and was not included in chromosomal abnormalities. At least two metaphases of each participant were karyotyped. Chromosome interpretation and designation was performed according to ISCN.

6.1.1. Metaphase chromosome preparation

The peripheral venous blood samples (1–3 ml) were collected from each participant into the sterile heparinised test tubes.

Procedure: 1. Preparation of cell culture (by Moorhead et al., 1960):
2–3 drops of blood were added to cell culture medium consisting of 4.25 ml of medium RPMI-1640, 0.75 ml of 10% foetal calf serum (FCS), 0.05 ml Phytohemagglutinin-M (PHA-M) (Gibco, Invitrogen, Rockville, MD) and incubated for 72 hours at 37°C.

The first mitoses occurred in the culture in 38–40 hours of incubation, and the maximum proliferation rate has been measured at 71–96 h. Using the 72 h culture second or third mitoses were analysed (Moorhead et al., 1960; Vogel, 1979; Therman, 1993).

2. The cell division was stopped at metaphase by the addition of colchicine to the culture (Schwarzacher, 1974). Colchicine arrested mitotic cultured cells in metaphase due to the block of microtubule assembly by binding to the tubulin heterodimer and thus inhibits spindle formation.

3. Pre-warmed at 37° C hypotonic solution of 0.075 M potassium chloride was added to the sediment and incubated in water bath at the temperature of 37°C. Hypotonic treatment caused a swelling of the cells because of difference of salts concentrations. The nuclear membrane was destroyed and mitotic spindle was depolymerised by colchicine. The optimal time of hypotonic treatment varies for different cell types, so it was determined empirically.

4. Fixative solution, a mix of absolute methanol and glacial acetic acid at a ratio of 3:1, was added to the sediment. Methanol removed most of the cytoplasm proteins; acetic acid coagulated nucleoproteins and fixed the cells.

5. After centrifugation, chromosome slides were made. Some drops of the culture were dropped on clean slide. The cell membrane became disrupted and chromosomes spread out on the slide. After drying in air, the slides were checked for chromosome spreading and cytoplasm debris under a microscope.

6.2. Staining methods

Conventional banding techniques are used to uniformly stain chromosomes and leave the centromeres constricted, thus enabling the measurement of chromosome length, centromeric position and arm ratio. The most frequently used chromosome banding techniques are GTG and CBG (Table 5).

Table 5. Banding techniques and detected band patterns of chromosomes

Type	Band pattern	Methods	References
G-banding method (GTG)	Except for the constitutive heterochromatin regions of chromosome 1, 9, 16 and the Y, the dark Giemsa bands are precisely comparable to the fluorescent Q-band patterns. Chromosome pattern shows darkly stained AT-rich bands	Enzymatic pretreating of chromosomes by trypsin, dehydratation in 70° and in 96° alcohol, and staining with working Giemsa stain (Giemsa stain + Sörensen buffer)	Seabright, 1971; Sumner et al., 1971; Drets et al., 1971; Patil et al., 1971; Schnedl, 1971;
C-banding method (CBG)	Chromosome pattern shows darkly stained constitutive heterochromatin of the centromere, the short arms of the acrocentrics, the secondary constrictions of 1, 9, and 16, and the distal long arm of the Y	Hydrolysis by 0.2N HCl with following DNA denaturation by $Ba(OH)_2$; renaturation in 2x SSC; staining with Giemsa stain	Pardue et al., 1970; Arrighi et al., 1971
R-banding method or reverse Giemsa banding method (RHG), produces bands complementary to G-bands	Chromosome pattern shows darkly stained R bands (GC-rich) and pale G bands (AT-rich). Useful for analysing deletions or translocations that involve the telomeres of chromosomes	Treatment of slides in hot phosphate buffer (high temperature and low pH) with subsequent Giemsa or acridine orange staining	Dutrillaux and Lejeune, 1971 Gustashaw, 1991
Q-banding method (QFQ)	Distinguish of the Y chromosome, also Y bodies in interphase nuclei, and various polymorphisms involving satellites and centromeres of specific chromosomes	Chromosomes are treated with solution of a fluorescent stain quinacrine	Caspersson et al., 1970 Sumner, 1972
AgNOR-staining (Silver Nucleolus Organising Region Staining)	Chromosome pattern shows darkly stained nucleolar organising regions (NOR), the secondary constrictions (stalks) of acrocentric chromosomes	Chromosomes are treated with silver nitrate solution	Howell et al., 1973 Bloom et al., 1976 Lay et al., 1978

6.2.1. Giemsa-banding method (GTG)

GTG with the use of trypsin for chromosome pretreating was first described by Seabright in 1971 (Table 5). Each homologous chromosome pair has a unique pattern of G-bands, enabling recognition of particular chromosomes.

Chromosomes were treated with the enzyme trypsin to partially digest the proteins along the chromosomes. Time of trypsin treatment needs to be correlated to the length of the chromosomes. Very long, prometaphase chromosomes require longer trypsin time and longer staining time, whereas short chromosomes require a shorter exposure time to trypsin and to Giemsa. Trypsin activity was terminated by dehydratation in a series of 70° and 96° alcohol followed by staining with Giemsa stain. The Giemsa stain, specific for the phosphate group of DNA, binds to the exposed DNA. The working solution of the Giemsa stain was prepared from the Giemsa stock stain and Sörensen phosphate buffer (pH 6.8). The staining results in a pattern of dark bands rich in adenine (A) and thymine (T), and light bands.

6.2.2. C-banding method (CBG)

CBG or constitutive heterochromatin banding method was firstly described in 1971 by Arrighi for banding of heterochromatin in human chromosomes. These regions stain darkly after treatment of the slide with 0.2N HCl solution, incubation in supersaturated Ba(OH)2 solution (50ºC) and incubation in salt-solution (60ºC) followed by staining with Giemsa stain.

Most of the DNA is denatured or extracted by treatment with alkali, acid, salt, or heat. Only heterochromatic regions close to the centromeres and rich in satellite DNA can stain. Different methods stain heterochromatic regions differently (Table 6).

Table 6. Comparison of staining methods for heterochromatic regions (by Shaw, 1973)

Staining method	1qh	9qh	16qh	Distal Yq
C-band (CBG)	+	+	+	+
G-band (GTG)	+	–	+	Variable
Q-band (QFQ)	–	–	–	+
R-band (FHG)	–	+	–	+

6.2.3. Fluorescent in situ hybridization (FISH)

The first time, the FISH method was used by Schmickel in 1986. This method involves the use of a DNA probe set or a single probe specific for an individual chromosome or chromosomal region for rapid diagnosis of a suspected

chromosomal abnormality or gene mapping. FISH is a cytogenetic technique, in which a labelled chromosome-specific DNA segment (probe) is incubated with metaphase, prophase or interphase chromosomes while hybridizing to a homologous fragment of DNA. Direct labelled FISH uses probes that have been prelabelled with a specific fluorophore, allowing the fluorescent signal to be bound to the target in a single hybridization step. The signal is visualised under fluorescence microscope. There are a lot of different direct DNA probes in use: whole chromosome painting, locus-specific and alphoid centromeric DNA probes. They have been used for identification of chromosomal regions according to particular interest.

In our study both whole chromosome painting probes (for chromosomes 5, 7, 10, 13, 16 and 21) and locus specific probes (for chromosomes 9, 13, 14, 15, 21, and 22) from Cytocell and Vysis were used. FISH procedures were performed according to manufacturer's instructions.

6.3. Analyses of alpha-1 antitrypsin serum level and phenotypes

Alpha-1 antitrypsin, the most important protease inhibitor, is a highly polymorphic plasma protein. The AAT level from serum in infertile men with Klinefelter's syndrome was determined by latex-enhanced immunoturbidimetric assay (reference value was 0.9–2.0 g/l). The AAT phenotype was defined by isoelectric focusing on ultra thin (0.25 mm) agarose gel layer (pH range 4.2–4.9) (Qureshi and Punnett, 1982). The AAT phenotype data of random population sample of similar genetic background (n=1422 persons) were used as controls (Uibo et al., 1991).

6.3.1. Latex-enhanced immunoturbidimetric assay

Serum sample (50 µl) was added to standard cassette with reagents and antibodies for the determination of alpha-1 antitrypsin level (Roche, Diagnostics) and put into a Cobas ® Integra 400 plus autoanalyser. Antibodies against AAT, which have been bound to the surface of the latex particles, form aggregates when a polyvalent antigen (ATT) is present. The intensity of a beam of light transmitted through the sample depends on the level of ATT in the serum and can be measured by autoanalyser.

6.3.2. Isoelectric focusing in agarose

Alpha-1 antitrypsin phenotypes were determined by isoelectric focusing (IEF) with carrier pharmalytes (Pharmacia, Sweden) on ultra thin (0.25 mm) agarose gel layer (pH range 4.2–4.9). Agarose gels were made of dry agarose powder (IsoGel®, FMC Corp.) dissolved in de-ionised water. Samples of 5 µl serum

were applied with filter paper pieces 2 cm from the cathode strip. A mixture of 0.025 M aspartic acid and 0.025 M glutamic acid was used for the anode electrolyte and 0.5 M glycine for the cathode electrolyte. Separation was carried out in an LKB 2117 Multicolor Electrophoresis Unit at 10 ºC for 4 h. The filter paper pieces were removed after 1 h. After focusing, gels were put into a fixing solution (mixture of sulfosalicylic acid, methanol and distilled water) for protein fixation. Finally they were stained with Coomassie Brilliant Blue R-250.

7. Statistical analyses

Student's independent t-test, chi-square test and descriptive analyses were applied to the statistical analyses. The differences between the compared groups considered statistically significant in all cases at $P<0.05$.

RESULTS

8. Cytogenetic analyses in infertile males (Paper I–VI)

8.1. Chromosomal alterations in infertile males

A total 90 infertile males were analysed cytogenetically (see 5.). From them 32 men had azoospermia and 58 males were oligozoospermics (including 53 men with severe oligozoospermia). As a control group, 30 healthy fertile men who had at least two children were studied. General view of chromosomal alterations found in infertile men and in controls is presented in Table 7.

Table 7. Chromosomal alterations in infertile males and in the control group, n(%)

Patients	Autosomal abnormalities	Sex chromosomal abnormalities	Major chromosomal abnormalities Total	Chromosomal variants	Total alterations
Infertile men (n=90)	7(7.8)	5(5.6)	12(13.4)	34(37.8)	43(47.8)
- Azoo-spermics (n=32)	1 (3.1)	4 (12.5)	5 (15.6)	13 (40.6)	16 (50)
- Oligo-zoospermics (n=58)	6 (10.3)	1 (1.7)	7 (12.1)	21 (36.2)	27 (46.6)
Control group (n=30)	–	–	–	13(43.3)	13(43.3)

8.2. Major chromosomal abnormalities and single cell pathology in infertile males in total

Incidence of major chromosomal abnormalities in infertile males was 13.4%, from which 7.8% were autosomal abnormalities and 5.6% were sex chromosome abnormalities (Table 7). In our study all of autosomal abnormalities were structural type, and all of sex chromosome abnormalities were numerical type.

In autosomal structural abnormalities different chromosomes were involved, and were predominantly translocations (5.6%, 5 cases) (Table 8). Two patients had sSMC.

Table 8. Autosomal abnormalities classified in term of sperm density in infertile men n(%)

Patients	Translo-cation	Marker-chromo-some	Total	Karyotype
Azoospermics (n=32)	1(3.1)		1(3.1)	45,XY,rob(13;14)(q10;q10)
Oligozoo-spermics (n=58)	4(6.9)		6 (10.3)	46,XY,t(10;15)(p11.1;q11.1) 46,XY,t(6;13)(p21.1;p11.2) mos 46,XY,t(1;15)(p34.1;q26.3)[3]/46,XY[97] 46,XY,t(7;16)(q21.2;p13.3)
		2(3.5)		mos 47,XY,+inv dup(22)[94]/ 48,XY,+inv dup(22),+21[3]/ 46,XY,t(14;21),+inv dup(22)[3] mos 47,XY,+mar[3]/46,XY[97]
Total (n=90)	5 (5.6)	2 (2.2)	7(7.8)	
Control group (n=30)	–	–	–	–

Sex chromosome abnormalities were found in 5 patients (5.6%) (Table 9). Four males had karyotype 47,XXY (Klinefelter's syndrome) and one was a mosaic form with karyotype 47,XXY[92]/46,XY[8].

Table 9. Sex chromosome abnormalities, classified in term of sperm density in infertile men, n(%)

Patients	Numerical abnormalities	Karyotype
Azoospermics (n=32)	4(12.5)	47,XXY (4 patients)
Oligozoospermics (n=58)	1(1.7)	mos 47,XXY[92]/46,XY[8]
Total (n=90)	5 (5.6)	
Control group (n=30)	–	–

Single cell pathology was found in 10% of infertile men, especially in azoospermia group (12.5%) (Table 10).

Table 10. Single cell pathology (SCP) in infertile azoospermic and oligozoospermic men, and in the control group, n(%)

Patients	SCP (%)	Karyotype
Azoospermics (n=32)	4(12.5)	46,XY,dup(8qter) 45,XY,t(6;13)(q15;q34) 47,XY,+17 45,XY,t(11;9)(q22.2;p23)
Oligozoospermics (n=58)	5(8.6)	47,XY,+mar 46,XY,inv(9)(p11.2q13) 46,XY,del(7q) 46,XY,del(2)(q13) 47,XY,+6
Total in infertile men n=90)	9 (10)	
Control group (n=30)	1(3.3)	47,XYY

8.3. Major chromosomal abnormalities in males with azoospermia and oligozoospermia. Characteristics of patients

The incidence of major chromosomal abnormalities was higher both in 32 men with azoospermia (15.6%) and in 58 men with oligozoospermia (12.1%) (Table 7) compared to controls.

8.3.1. Males with azoospermia

5 males of 32 men with azoospermia had major chromosomal abnormalities (Table 11).

Table 11. Characteristics of patients with azoospermia

Patients	Karyotype	Age, years	BMI	Small testes Dex/Sin (ml)	Gynaeco-mastia	Varicocele
Patient 1	rob t(13;14)	36	23.4	No date	–	+
Patient 2	47,XXY	21	23.7	+ 2/2	–	–
Patient 3	47,XXY	31	25.4	+ 10/7	–	–
Patient 4	47,XXY	29	19.9	+ 6/5	–	+
Patient 5	47,XXY	24	27.8	+ 3/3	–	–

All the patients with the classical form of Klinefelter's syndrome and 47,XXY karyotype were infertile, and have several other clinical signs with variable frequency.

Autosomal abnormalities were found in one man (*Patient 1*) with azoospermia (3.1%) (Table 11).

Patient 1: He was a 36-year old infertile man with normal BMI and had a Robertsonian translocation between chromosomes 13 and 14 (Figure 1) and polymorphic chromosomal variants 9qh+ and 13pss. The karyotype was: 45,XY,t(13;14)(q10;q10).

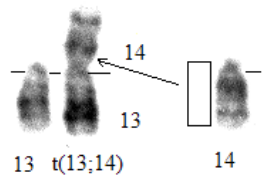

Figure 1. Robertsonian translocation between acrocentric chromosomes 13 and 14.

Sex chromosome abnormalities were revealed in 4 patients (12.5%) (Table 11).

Patients 2–5: All of the patients had 47,XXY karyotype, classical form of Klinefelter's syndrome (Figure 2). Patient 4 had also multiple polymorphic variants: 1qh+,16qh-,Yqh-.
All of the patients had small testes and were azoospermics.

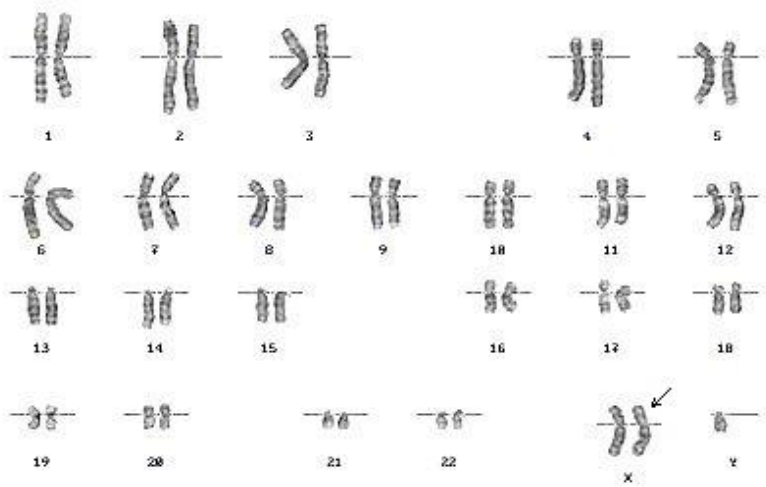

Figure 2. 47,XXY karyotype –Klinefelter's syndrome

8.3.2. Males with oligozoospermia

7 males of 58 men with oligozoospermia had major chromosomal abnormalities (Table 12).

Autosomal structural abnormalities were revealed in 6 patients (10.3%) (Table 8, 12). Translocations (6.9%) were found in four males with severely depressed spermatogenesis (sperm count ≤5 mln/mL). Their physical and mental development was normal.

Patient 6: He was a 34-year old infertile man, slightly overweight (BMI 25.4). Cytogenetic analysis revealed translocation between chromosomes 10 and 15 in all cells studied. The finding was subsequently confirmed by the FISH method (Mikelsaar et al., 2007). The karyotype was: 46,XY,t(10;15) (p11.1; q11.1) (Figure 3).

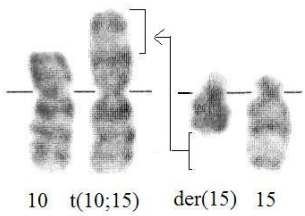

Figure 3. Translocation t(10;15).

Table 12. Characteristics of patients with oligozoospermia

Patients	Chromosomal abnormality	Sperm density, mln/mL	Age, years	BMI	Small testes, Dex/sin (ml)	Gynaeco-mastia	Varico-cele
Patient 6	t(10;15)	5	34	25.4	No data	–	–
Patient 7	t(6;13)	0.01	28	No data	–	–	–
Patient 8	mos t(1;15) (3%)	3	36	24.8	–	–	+
Patient 9	t(7;16)	2.6	38	27.7	–	–	–
Patient 10	sSMC inv dup(22)	2.9	32	No data	–	–	–
Patient 11	mos sSMC (3%)	13	29	No data	–	–	–
Patient 12	mos 47,XXY (92%)	0.005	27	24.2	+ 4/2	–	–

Patient 7: He was a 28-year old infertile but otherwise healthy man. He had severe oligozoospermia (sperm count 0.01 mln/mL, sperm motility 0%). Chromosomal analysis showed non-reciprocal translocation between chromosomes 6 and 13 (Table 12). The karyotype was: 46,XY,t(6;13)(p21.1;q11.2) (Figure 4).

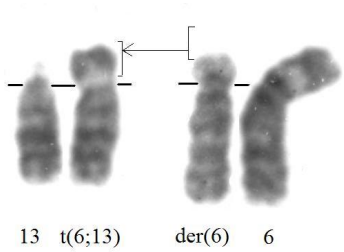

Figure 4. Translocation t(6;13).

Patient 8: He was a 36-year old man (Table 12). He had severe oligozoospermia (sperm count 3mln/mL) and abnormal sperm quality (motility A+B=32%, grade A=9%; normal morphology 0%). The karyotype showed non-reciprocal translocation between chromosomes 1 and 15 in 3% of cells. The karyotype was: mos 46,XY,t(1;15)(p34.1;q26.3)[3]/46,XY[97] (Figure 5).

Figure 5. Translocation t(1;15).

Patient 9 **(Paper II)**. The patient was a 38-year-old infertile man (Table 12). To assess the causes of the infertility of 10 year's duration, the patient underwent physical examination, semen analysis, and chromosome analyses. His physical examination revealed a healthy male. The size and consistency of both the testes and epididymises were normal. The results of two different semen analyses of the patient showed severe oligoasthenoteratozoospermia with sperm count

ranging from $2x10^6$/mL to 5 $x10^6$/mL, sperm hypomotility (A+B=35%), and abnormal sperm morphology. The incidence of sperm head defects was 98%.

Chromosomal analysis of 30 peripheral blood lymphocyte metaphases using GTG and CBG banding methods revealed a translocation t(7;16) with breakpoints at 7q21.2 and 16p13.3 in all the cells examined (Fig. 6A). FISH method with chromosome 7–specific (WCP7 green) DNA painting probe and a chromosome 16–specific (WCP16 orange) DNA painting probe (Vysis, Downers Grove, IL) were used for the identification of the structure of this translocation. The results of FISH confirmed the presence of a reciprocal translocation with translocated 7q material to 16p, and with reciprocally translocated the small terminal part of 16p to 7q (Fig. 6B).

In addition, a CBG banded karyogram revealed inversion of chromosome 9, which is thought to be a normal variant and does not affect fertility parameters in men (Hamer et al., 1981). The patient's karyotype was interpreted as 46,XY,t(7;16)(q21.2; p13.3),inv(9)(p11q13).

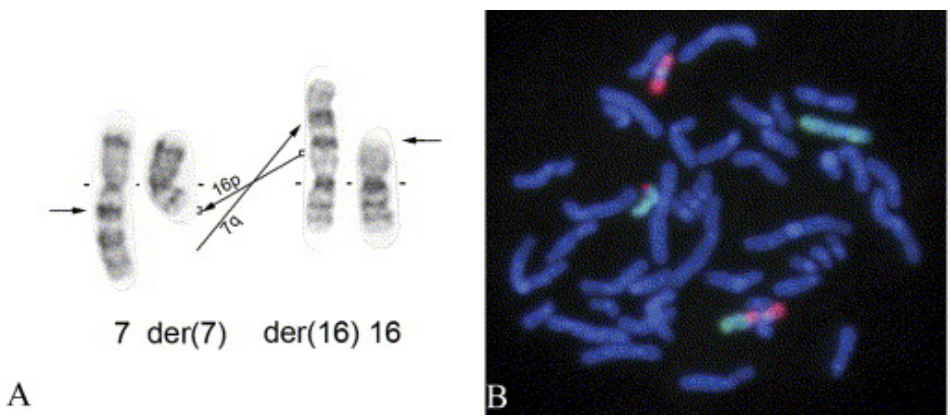

Figure 6. (A) Partial karyotype of GTG banded chromosomes 7 and 16, showing the t(7;16)(q21.2;p13.3).(B) FISH using chromosome 7–specific (WCP7 green) and chromosome 16–specific (WCP16 orange) DNA painting probes (Vysis) identified the reciprocal t(7;16).

The problems connected with the association of detected chromosomal abnormality with infertility will be discussed in details (see 10.1).

Small supernumerary marker chromosomes (sSMCs) were found in two men (3.5%) with oligozoospermia (Table 8). Both supernumerary marker chromosomes were in mosaic forms.

Patient 10. **(Paper IV)** (see 10.2) (Table 12). He was a 32-year old infertile man with severe oligoasthenoteratozoospermia with a sperm count of 2.9 mln/mL, sperm hypomotility (A+B=10%) and no normal sperm morphology. The volume of the right testis was 18 ml and that of the left testis was 14 ml (normal average testicular volume is 18 ml) (Nieschlag and Behre, 2001). His physical examination revealed high blood pressure but otherwise a healthy male with no smell disorders. The results of repeated hormone analyses measured by the chemiluminescence method showed hypogonadotropic hypogonadism (HH) with a low level of serum testosterone (3.8–4.3 nmol/l) and low-normal levels of follicle-stimulating hormone and luteinizing hormones being both 1.5 μU/ml (normal ranges: 6.9–28.1 nmol/l, 0.7–11.1 μU/ml, and 0.8–7.7 μU/ml, respectively).

Cytogenetic analyses revealed sSMC both in lymphocyte and fibroblast cultures in 100% of cells (Figure 7). However, in blood, there were 3% of cells with trisomy 21 and t(14;21) in other 3% of cells.

The karyotype was: mos 47,XY+mar[94]/46,XY,t(14;21),+mar[3]/48,XY+mar+21[3] in blood. The karyotype from the skin fibroblasts was 47,XY,+mar.

FISH with five different chromosome-specific and locus-specific DNA probes: P5015, P5090, P5032 (Oncor), LSI SNRPN (Vysis Inc.) and probe no. 1690612 (Boehringer) indicated that the sSMC derived from the chromosome 22 being inv dup(22). With the use of LSI KAL Spectrum Orange/CEP X Spectrum Green Control Probe (Vysis), no deletions in the KAL-1 gene were found. No Y-chromosomal microdeletion could be found with the deletion analysis of the regions of AZF loci (AZFa, AZFb and AZFc).

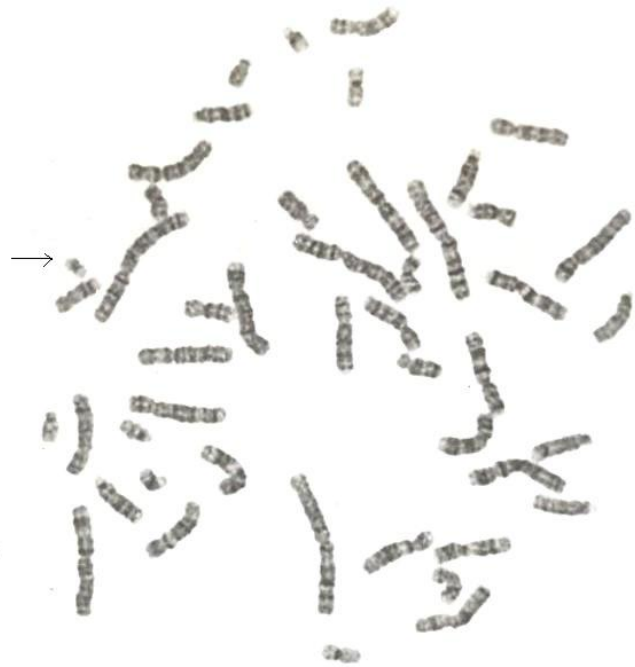

Figure 7. Supernumerary marker chromosome in Giemsa-banded metaphase.
Patient 11: He was a 29-year old infertile man with oligozoospermia (sperm count 13 mln/mL) (Table 12). Chromosomal study revealed a mosaic form of sSMC (origin not identified) only in 3% of blood cells. His karyotype was: 47,XY,+mar[3]/46,XY [97].

Sex chromosome abnormalities were found in one patient (1.7%)
Patient 12. He was a 27-year old healthy man but infertile with oligozoospermia (sperm count 0.005 mln/mL) and abnormal sperm quality (immobile spermatozoa A+B=0%, normal morphology 0%). Testes were small with a volume on the right side of 4 ml and 2 ml on the left side. Cytogenetic analyses showed an extra X chromosome in 92% of blood cells, and so he had a mosaic form of Klinefelter's syndrome (Table 12). The karyotype: 47,XXY[92]/ 46,XY[8].

8.4. Polymorphic chromosomal variants in infertile males

Polymorphic chromosomal variants (Figure 8) were found in 34 infertile males (37.8%) which incidence was similar to that in 13 fertile men from the control group (43.3%) (P>0.05). Autosomal chromosomal variants were observed more frequently than sex chromosomal variants (Table 13).

Table 13. Autosomal and sex chromosome polymorphisms, classified in term of sperm density in infertile men and in the control group, n(%)

Patients	Chromosomal polymorphisms in total	
Azoospermics (n=32)	13(40.6)	1qh- 1qh+,9qh+ (2 patients) 1qh+,16qh-,Yqh- 9qh+ 9qh+,9qh-,inv(9) 9qh+,13pss 9qh+,16qh+ 9qh+,inv(9),Yqh+ 9qh-,21ps+ inv(9) (3 patients)
Oligozoospermics (n=58)	21(36.2)	1qh+ (2 patients) 1qh+,Yqh+ 1qh-,9qh+,Yqh+ inv(1) 9qh+ (8 patients) 9qh+,16qh- 9qh+,16qh+ inv(9) (4 patients) Yqh+ (2 patients)
Total (n=90)	34 (37.8)	
Control group (n=30)	13(43.3)	1qh+,9qh+ 1qh+,inv(1) inv(1),9qh- 3qh+, 9qh+, 9qh- 9qh+ (3 patients) 9qh-,16qh-,Yqh- 9qh+,Yqh+ inv(9) 14pss 14pss,21ps+ 22ps+

The most frequent chromosomal variant of autosomes was 9qh+ found in 18 infertile males (20%) and in 6 fertile men (20%). Inv(9) was found in 8 (8.9%) infertile men and in one man (3.3%) from control group (P>0.05). The most frequent sex chromosome variant was Yqh+ found in five (5.6%) infertile men and in one man (3.3%) of control fertile men (P>0.05).

In the azoospermia group the total incidence of chromosomal variants was 40.6% that is similar to this in the oligozoospermia group (36.2%) and controls (43.3%) (P>0.05). In the azoospermia group the incidences of variants 9qh- (6.3%), inv(9) (15.6%) and satellite variants (6.3%) were higher than those in

the oligozoospermia group (0%, 6.9%, and 6.3%, respectively). Contrarily, in the oligozoospemia group Yqh+ variant (6.9%) was more frequent in comparison with that in the azoospermia group (3.1%) (Table 14).

In three infertile men major chromosomal abnormalities and chromosomal variants were both found. Two of them were in the azoospermia group:

47,XXY,1qh+,16qh-,Yqh- and
45,XY,t(13;14)(q10;q10),9qh+,13pss

and one was a man with oligozoospermia:

46,XY,t(7;16)(q21.2;p13.3),inv(9)(p11q13)

Table 14. Incidence of polymorphic variants in infertile males with azoo- and oligozoospermia, and in the control group

Polymorphic variants	Azoospermics, n=32 (%)	Oligozoospermics, n=58 (%)	Total in infertile men, n=90 (%)	Controls, n=30 (%)
Total variants of chromosome 1	4 (12.5)	4 (6.9)	8 (8.9)	3 (10)
1qh+	3 (9.4)	3 (5.3)	6 (6.7)	2 (6.7)
Inv(1)	0 (0)	1 (1.7)	1 (1.1)	2 (6.7)
1qh-	1 (3.1)	1 (1.7)	2 (2.2)	0 (0)
Total variants of chromosome 9	11 (34.4)	15 (25.9)	26 (28.9)	8 (30)
9qh+	7 (21.9)	11 (19)	18 (20.0)	6 (20)
inv(9)	5 (15.6)	4 (6.9)	9 (10.0)	1 (3.3)
9qh-	2 (6.3)	0 (0)	2 (2.2)	3 (10)
Total variants of chromosome 16	2 (6.3)	2 (3.4)	4 (4.4)	1 (3.3)
16qh+	1 (3.1)	1 (1.7)	2 (2.2)	0 (0)
16qh-	1 (3.1)	1 (1.7)	2 (2.2)	1 (3.3)
Total variants of the Y chromosome	2 (6.3)	4 (6.9)	6 (6.7)	2 (6.7)
Yqh+	1 (3.1)	4 (6.9)	5 (5.6)	1 (3.3)
Yqh-	1 (3.1)	0 (0)	1 (1.1)	1 (3.3)
Satellites	2 (6.3)	0 (0)	2 (2.2)	3 (10)

The differences between the groups were not statistically significant ($P>0.05$).

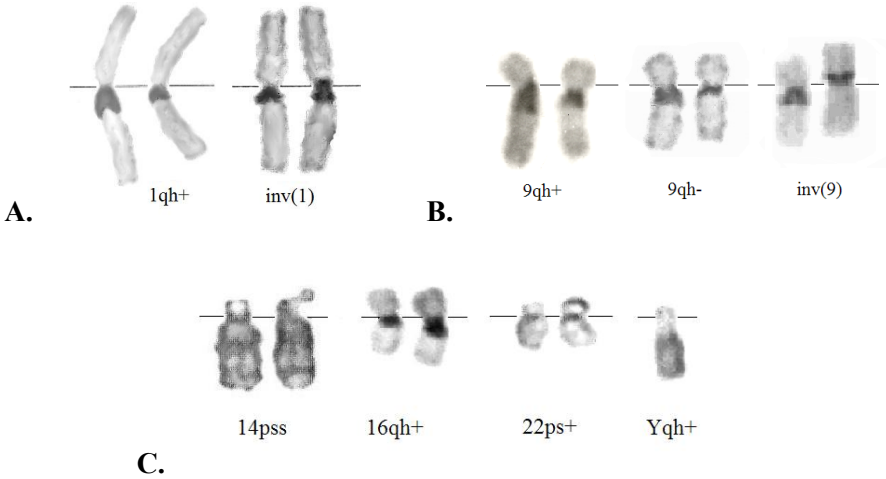

Figure 8. Polymorphic chromosomal variants. A. Variants of chromosome 1. B.Variants of chromosome 9. C. Variants of chromosomes 14, 16, 22 and Y.

8.5. Alpha-1 antitrypsin serum level and phenotypes in Klinefelter's syndrome (Paper III)

We have studied 13 infertile patients with Klinefelter's syndrome with one or more other disorders (Table 15). The pathogenesis of clinical signs, especially azoospermia, is not fully understood. We conducted a pilot study to explain if there was any association between the Klinefelter's syndrome and alpha-1 antitrypsin (ATT) deficiency.

The AAT level of our patients was 0.74–1.48 g/l (controls range 0.9–2.0 g/l) with a mean value of 1.25 g/l (controls value 1.45 g/l). Four individuals (3, 5, 7, 10) had AAT levels lower than the mean value of the patient group (1.25 g/l). AAT phenotypes, revealed by isoelectric focusing, were Pi M types in 11 subjects. Two of the 13 patients had a phenotype with AAT deficiency. One patient (no. 10) had the Pi XZ phenotype. The second (no. 13) had the Pi M_10 phenotype and an AAT level of 1.44 g/l. This patient was suffering from infected leg ulcers.

Table 15. Patients with Klinefelter's syndrome, their serum AAT levels, AAT phenotypes and specific diseases (ICD-10) (Ruth Mikelsaar et al.) (Paper III).

Patient	AAT g/l	AAT Pi	Smoker	Pneumo-nia (J18)	Bron-chitis (J20)	Allergy (J30.1)	↑blood pres-sure (I10)	Skin disease (L20.9; L97)	Kidney Disease (N10)
1.	1.39	M_1M_3	+	+	+	+		+	
2.	1.34	M_1M_1	–	+	–				
3.	1.17	M_1M_1	+	+	–				
4.	1.29	M_1M_2	–	–	–		+		
5.	1.18	M_1M_1	–	+	+	+			
6.	1.48	M_1M_1	+	–	–		+		
7.	1.09	M_1M_1	+	–	+				
8.	1.32	M_1M_1	+	+	–		+		
9.	1.32	M_1M_1	+	+	+				
10.	0.74	XZ	+	+	+				+
11.	1.23	M_1M_1	+	–	–	+			
12.	1.34	M_1M_1	+	–	+				
13.	1.44	M_10	+	–	–			+	

AAT-alpha-1 antitrypsin

DISCUSSION

9. Cytogenetic analyses in infertile males

As reported in the literature, in half of couples with unsuccessful pregnancy the cause of infertility is male-related, and of them in about 30% genetic factors should be considered. In this study 90 infertile males and 30 control fertile males have been analysed in relation to chromosomal abnormalities.

9.1. Chromosomal alterations in total

Incidence of chromosome alterations, including major chromosomal abnormalities and polymorphic chromosomal variants, was high in infertile males (47.8%) in our study (Table 16). However, the difference between infertile males and controls was significant ($p<0.05$) only for major chromosomal abnormalities, and not for chromosomal variants ($p>0.05$). Major chromosomal abnormalities were not found in fertile control males.

The incidence of chromosomal alterations in our study is about two times higher than that given in the literature by some authors (12.6–28.3%) (Nielsen 1988; Nakamura et al., 2001; Wiland et al., 2002), but is similar with the others (47.7% and 57.1%, respectively) (Nagvenkar et al., 2005; Penna-Videau, 2001). The observed differences in the results may be caused by the different selection criteria.

1) In our study the number of patients (n=90) was not very large, but sufficient to compare our results with those of other authors and assess the importance of chromosomal abnormalities in male infertility. 2) The selection of patients in our study was very strict. Most of our patients had severely impaired spermatogenesis (only 5 men of 90 had sperm count > 5 mln/mL). The same criteria to the selection of patients were only in study of Nagvenkar (2005), while other authors have included all infertile males with abnormal spermiogram (sperm count < 20 mln/mL) (Penna-Videau et al., 2001; Wiland et al., 2002). Some studies have included only those infertile men who abnormal spermiogram further normalised (Nakamura et al., 2001; Nielsen 1988) (Table 16). 3) It is possible that also an ethnic factor also has some influence on the observed differences. There are data that the incidences of chromosomal variants vary between different countries (Bhasin, 2005).

Table 16. Chromosomal alterations in infertile men, n (%)

Author	No of patients	Country	Major chromosomal abnormalities, n(%)	Chromosomal variants, n(%)	Total chromosomal alterations, n(%)
Nagvenkar et al., 2005	88	India	9(10.2)	33(37.5)	42(47.7)
Nakamura et al., 2001	1790	Japan	138 (7.7)	87 (4.9)	225 (12.6)
Nielsen, 1988	212	Denmark	19 (9)	13 (6.1)	31 (14.6)*
Penna-Videau et al., 2001	84	Spain	19 (22.6)	29 (34.5)	48 (57.1)
Wiland et al., 2002	60	Poland	2 (3.3)	15 (25)	17 (28.3)
Total	**2234**		**187 (8.4)**	**177 (7.9)**	**363 (16.2)**
Our study, (infertile males) Paper I 2006	90	Estonia	12 (13.4)	34 (37.8)	43 (47.8)**
Controls	30		0 (0)	13 (43.3)	13 (43.3)

* One patient had both a major chromosomal abnormality and polymorphic variant;
** Three patients had both a major chromosomal abnormality and polymorphic variants

9.2. Major chromosomal abnormalities in infertile males

The incidence of major chromosomal abnormalities is very low in large studies of normal newborns and normal fertile males (Table 17). Although our control group is a small one, the incidence of major chromosomal abnormalities (0%) in this group is comparable to the results obtained in larger groups (0.3–0.38%).

Table 17. Incidence of major chromosomal abnormalities in controls, n(%).

Author	No of patients	Sex chromosome abnormalities n(%)	Autosomal abnormalities n(%)	Major chromosomal abnormalities n(%)
Our study, Paper I 2006	30	0 (0)	0 (0)	0 (0)
Newborn infants Van Assche et al., 1996	94 466	131 (0.14)	232 (0.25)	366 (0.38)
Normal fertile males Ravel et al., 2006	10 202	14 (0.14)	24 (0.24)	38 (0.37)
Normal fertile males Foresta et al., 2005	295	–	–	1 (0.3)

The total incidence of major chromosome abnormalities in infertile males in our study was 13.4%, which is significantly ($P < 0.05$) higher than that in the control group (0%) (Table 18) and is similar to the literature data (varying from 2.4% to 16.4% (Van Assche et al., 1996; Pandiyan et al., 1996; Gekas et al., 2001; Foresta et al., 2005; Elghezal et al., 2006; Chiang et al., 2000; Ng et al., 2009; Koşar et al., 2010; Yatsenko et al., 2010).

The incidences of autosomal abnormalities (7.8%) and sex chromosome abnormalities (5.6%) were also coincidental with those of other authors (Table 18).

Although not statistically significantly, autosomal chromosome abnormalities (7.8%) in our study predominated over sex chromosome abnormalities (5.6%) in infertile men, as has been shown also by some other authors, but there are also contradictive results (Table 18). This may be caused by differences in the criteria of selection of patients to the study. In the literature there are data that autosomal chromosome abnormalities cause more frequently oligozoospemia than azoospemia (Yoshida et al., 1996). We have similar data (see Table 8 and 9.2.1).

Table 18. Major chromosomal abnormalities in infertile males, n(%).

Author	No of patients	Sex chromosome abnormalities n(%)	Autosomal abnormalities n(%)	Major chromosomal abnormalities n(%)
Bertini et al., 2006	435	18 (4.1)	5 (1.1)	23 (5.2)
Bonaccorsi et al., 1997	103	4 (3.9)	7 (6.8)	11 (10.6)
Bourrouillou et al., 1985	952	65 (6.8)	33 (3.4)	98 (10.3)
Chiang et al., 2000	220	27 (12.3)	9 (4.1)	36 (16.4)
Cruger et al., 2003	392	11 (2.8)	5 (1.3)	16 (4.1)
Dohle et al., 2002	150	9 (6)	7 (4.7)	16 (10.6)
Elghezal et al., 2006	1000	91 (9.1)	44 (4.4)	135 (13.5)
Foresta et al., 2005	750	32 (4.3)	10 (1.3)	42 (5.6)
Gekas et al., 2001	2196	82 (3.7)	52 (2.4)	134 (6.1)
Micic et al., 1984	820	45 (5.5)	9 (1.1)	60 (7.3)
Nagvenkar et al., 2005	88	4 (4.5)	5 (5.7)	9 (10.2)
Nakamura et al., 2001	1790	99 (5.5)	39 (2.2)	138 (7.7)
Nielsen., 1988	212	15 (7.1)	4 (1.9)	19 (9)
Pandiyan et al., 1996	1210	27 (2.2)	17 (1.4)	44 (3.6)
Penna-Videau et al., 2001	84	19 (22.6)	0 (0)	19 (22.6)
Rao et al., 2004	251	10 (4)	18 (7.2)	28 (11.2)
Van Assche et al., 1996	568	2 (0.35)	12 (2.1)	14 (2.4)
Van der Ven et al., 1997	204	2 (0.98)	4 (2)	6 (2.98)
Vicdan et al., 2004	208	2 (1)	5 (2.4)	7 (3.4)
Wiland et al., 2002	60	0 (0)	2 (3.3)	2 (3.3)
Total	**11781**	**564 (4.8)**	**287 (2.4)**	**866 (7.4)**
Our study, 2006 Paper I	90	5 (5.6)	7 (7.8)	12 (13.4)

9.2.1. Autosomal abnormalities

The incidence of autosomal abnormalities in our study was 7.8%, which was higher than that (1.1–5.7%) given in the literature of most authors (Table 18), but similar to the data from some others (6.8–7.2%) (Bonaccorci et al., 1997; Rao et al., 2004).

In our study all the major autosomal abnormalities were structural type involving different chromosomes (chromosomes 1, 6, 7, 10, 13, 14, 15, 16 and 22). Of 7 infertile males with autosomal abnormalities 5 had translocations and two had sSMCs.

Translocations. The incidences of reciprocal and non-reciprocal translocations (except rob) in our study were significantly higher in infertile males (4.4%) than in the control group (0%) ($p<0.05$) and about two times higher than that in the literature (1.3–1.6%) (Elghezal et al., 2006; Pauer et al., 1997).

From 5 translocations, there was one reciprocal t(7;16), 3 non-reciprocal translocations, which involved acrocentric chromosomes [t(6;13); t(1;15) and (10;15)], and one rob t(13;14). As the most frequent chromosome involved in rearrangements in infertile men is reported the chromosome 1 (Bugge et al., 2000; Bache et al., 2004), so we also found one patient with non-reciprocal translocation of this chromosome. Acrocentrics are the chromosomes, which are frequently involved in chromosomal rearrangements in infertile males. Thus it was also in our group of infertile males: of 5 translocations 4 have involved one or two acrocentrics. This problem needs further investigation.

The incidence of translocations (5.6%) in our study was higher than that given by some other authors (0.4–1.3%) (Stern et al., 1999; Foresta et al., 2005). This difference may be occur because by the differences of selected criteria of studied infertile males: more than half (53/90) of our patients had severe oligozoospermia.

Translocations may cause 1) synaptic failure around breakpoints (Bache et al., 2004; Oliver-Bonet et al., 2005; Martin, 2008). 2) Association of the translocation figure with the sex chromosomes causing XY-pairing failure. The main condition favouring contact between a trivalent or quadrivalent and the XY configuration at prophase of meiosis is asynapsis in one arm of the multivalent, which take place between the short arms of the acrocentrics (Johnson, 1998; Oliver-Bonet et al., 2005). 3) Frequent occurrence of an acrocentric chromosome in the translocation (see 3.1.1.) (Oliver-Bonet et al., 2005; Vialard et al., 2006).

Reciprocal translocations form quadrivalents in meiosis, which through impairment of chromosomal segregation can lead to reduced fertility, spontaneous abortions or birth defects, depending on the chromosomes involved and the nature of the translocation (Shah et al., 2003). Non-reciprocal translocations involving acrocentric chromosomes cause more severe spermatogenesis impairment because of a tendency of acrocentric chromosomes to associate with the sex body (Oliver-Bonet et al., 2005; Vialard et al., 2006).

Although Robertsonian translocation t(13;14) is the most frequent in infertile males, we found it only in one case (1.1%) that is similar to the literature data (1.0–2.3%). In meiosis, t(13;14) forms trivalent that may affect pairing of homologous chromosomes during the I meiotic division and cause male infertility (Testart et al., 1996; Thielemans et al., 1998; Elghezal et al., 2006).

Small supernumerary marker chromosomes (sSMCs). sSMCs are rare, especially in infertile males. In our study two supernumerary marker chromosomes were found with an incidence of 3.5% which is higher than in the control group (0%) and than that reported in infertile men (0.12%) in the literature (Elghezal et al., 2006). Males carrying a sSMC are often phenotypically normal. SMC may associate with the X-Y bivalent at meiotic prophase and cause male infertility through impairment of spermatogenesis due to meiotic arrest and instability resulting in maturation arrest on spermatocyte stage (Chandley et al., 1987; Johnson 1998).

9.2.2. Sex chromosome abnormalities

Incidence of sex chromosome abnormalities in our study was 5.6%, which coincides with the data of the literature, varying from 0.98% to 12.3% (Table 18). All sex chromosome abnormalities were numerical type with karyotype 47,XXY and one case (1.1%) in mosaic form 47,XXY/46,XY. It supports the opinion that they are the most frequent sex chromosome abnormality in infertile men. Incidence of KS in infertile males in our study is significantly higher than that in the control group ($p<0.05$) but similar with incidence of KS in sterile men (1–2%) in the literature (Brugo-Olmedo et al.,2000).

Patients with KS have impaired spermatogenesis, with severe oligozoo- or azoospermia, causing infertility. This is caused by lethal gene dosage introduced into cells by an additional X chromosome, which does not permit the development of Sertoli cells and survival of germ cells in the testis, resulting in azoospermia due to advanced germ cell atresia and aplasia (Johnson, 1998; Thielemans et al., 1998) (see 3.2.1.).

9.2.3. Single cell pathology

Single cell pathology is used to consider as an artefact (Gekas et al., 2001; Scholtes et al., 1998). However, we have found higher percentage of single cell pathology in infertile men (10%), especially in the azoospermia group (12.5%) compared to that in the control group (3.3%). Some authors have reported that a high frequency of single cell pathology in infertile males might be a very mild form of undetected mosaicism or shows that germ cells are affected as well (Peschka et al., 1999).

9.3. Chromosomal abnormalities in males with azoospermia and oligozoospermia

In our study the incidence of chromosomal alterations was 50% in men with azoospermia, 46.6% of men with oligozoospermia and 43.3% in the control group (Table 7) that is in agreement with the literature data. The high frequency of chromosomal alterations in azoo- and oligozoospermics was also reported by Penna-Videau (71% and 51%, respectively).

The incidences of major chromosomal abnormalities both in the azoospermia (15.6%) and oligozoospermia groups (12.1%) were significantly higher ($P < 0.05$) than that in the control group (0%). Incidence of chromosomal abnormalities in the azoospermia group in our study was similar to that reported in the literature (13.1–23.6%), while in the oligozoospermia group it was about two times higher than that reported by other authors (2.6–6.6%) (Rivas et al., 1987; Nielsen, 1988; Yoshida et al., 1996; Bonaccorsi et al., 1997; van der Ven et al., 1997; Johnson, 1998; Thielemans et al., 1998; Chiang et al., 2000; Dohle et al., 2002; Cruger et al., 2003; Rao et al., 2004; Vicdan et al., 2004; Dada et al., 2006; Elghezal et al., 2006). As the majority of the oligozoospermic patients in our study (49/58) had severe oligozoospermia, then this incidence (12.1%) (Table 7) is similar to that in the literature (10.6%) (Dohle et al., 2002). These findings may confirm the existence of inverse correlation between sperm count and incidence of chromosomal abnormalities, although the literature data of it are still contradictory.

The incidence of sex chromosome abnormalities (12.5%) in the azoospermia group was higher ($P<0.05$) than that in the oligozoospermia group (1.7%) (Table 7). In contrast, autosomal abnormalities were more frequent (10.3%) in the oligozoospermia group than in azoospermic males (3.1%) ($P>0.05$). All the autosomal abnormalities were structural type and all the sex chromosome abnormalities were numerical type in our study. This coincides with the data of the literature and suggests that sex chromosome abnormalities of numerical type depress spermatogenesis more than autosomal abnormalities of the structural type.

9.3.1. Azoospermia group

Autosomal abnormalities. Autosomal abnormalities were found in one patient (3.1%) with azoospermia. A similar frequency has also been shown by others (0.9–2.9%) (van der Ven et al., 1997; Johnson, 1998; Chiang et al., 2000; Dohle et al., 2002; Cruger et al., 2003; Rao et al., 2004; Vicdan et al., 2004; Foresta et al., 2005; Elghezal et al., 2006). This patient was a phenotypically normal but infertile male (*Patient 1*) with karyotype 45,XY,t(13;14)(q10;q10). He also had polymorphic variants 9qh+ and 13pss. He had an autosomal abnormality – Robertsonian translocation t(13q;14q), the incidence of which coincided with the literature data in azoospermics. Infertility in this patient might be caused by

the difficulties with pairing of homologous chromosomes forming trivalent during the I meiotic division. The association of acrocentric chromosomes with the X-Y body results in severe spermatogenic defects and significantly increases risk of unbalanced spermatozoa (7% to 40%), and miscarriages (Escudero et al., 2000; Frydman et al., 2001; Oliver-Bonet et al., 2005; Martin, 2008).

Polymorphic chromosomal variants 9qh+ and 13pss may also increase the risk of production of unbalanced gametes resulting in infertility or spontaneous abortions in partners. It is proposed that a heterochromatic block may cause nondisjunction of chromosomes and subsequently meiotic arrest (Yakin et al., 2005) (See also 9.4.).

Sex chromosome abnormalities. In the azoospermia group sex chromosome abnormalities were predominant over autosomal abnormalities (12.5% and 3.1%, respectively), which coincided with the literature data. All sex chromosome abnormalities were presented as 47,XXY (12.5%), which is similar to the literature data 7-13% (De Braekeleer and Dao, 1991; Yoshida et al., 1996; Brugo-Olmedo et al., 2000), and confirm that 47,XXY is the most frequent sex chromosome abnormality in infertile males with azoospermia (see also pages 21–22).

We have found four infertile males (*Patients 2–5*) with 47,XXY karyotype. They all had azoospermia and small testes that have also been reported in the literature. Contrarily to the literature, no gynaecomastia was observed in our patients. Infertility in these patients first of all is because of the direct harmful effect of an extra X chromosome causing lethal gene dosage effect in the cells in the testes resulting in azoospermia due to advanced germ cell atresia and aplasia (Thielemans et al., 1998). One of the patients (Patient 4) also had polymorphic chromosomal variants 1qh+,16qh- and Yqh-. Polymorphic chromosomal variants of chromosome 1 have been reported in a relationship to foetal wastage and recurrent miscarriage, whereas variant 1qh+ is associated with congenital malformations (Bhasin, 2005). Variant Yqh- has been reported in association with male infertility. Oppositely, variant 16qh- was not reported in infertile males.

9.3.2. Oligozoospermia group

Autosomal abnormalities. Autosomal abnormalities were found in 6 patients (10.3%) of oligozoospermic patients, which is similar to the literature data (1.3–10.2%), but significantly higher than that in our control group ($p<0.05$). Autosomal abnormalities in oligozoospermic patients are predominant over sex chromosome abnormalities (1.7%) and are about three times more frequent than in the azoospermic group (3.1%). This is in agreement with the literature data that carriers of autosomal chromosome abnormalities are at a higher risk of oligozoospermia (Yoshida et al., 1996). From 6 autosomal abnormalities 4 patients had translocations (6.9%) between different chromosomes (6,7,10,13, 14,15 and 16). Translocations involving an acrocentric chromosome have been

thought to be more harmful for the infertility of the carrier than translocations not involving acrocentric chromosomes because of the tendency of acrocentric chromosomes to associate with the sex body (Oliver-Bonet et al., 2005) (see also 3.1.1.). The incidence of translocations in the oligozoospermia group (6.9%) is similar to that reported in the literature in oligozoospermics (5.2%) (Chiang et al., 2000) but significantly higher than in our fertile control males. All oligozoospermic patients with autosomal abnormalities were childless, no gynaecomastia or other phenotypic abnormalities (Table 11).

In the present study, we have found one man (*Patient 6*) with karyotype 46,XY,t(10;15)(p11.1;q11.1) and severe depressed spermatogenesis (sperm count 5 mln/mL) (Mikelsaar et al., 2007). Non-reciprocal translocation with the involvement of an acrocentric chromosome may affect spermatogenesis in a different way. Synaptonemal disturbances or an association between quadrivalent and an X-Y body can result in destruction of the germ cell and cause meiotic arrest at the primary spermatocyte stage. The role of genes involved in spermatogenesis is also suggested. Interchromosomal effect depends on the type of structural reorganisation, the chromosomes and the chromosomal breakpoints (Blanco et al., 2000; Anton et al., 2002). Thus our patient is the first case of an infertile man with severe oligozoospermia described in t(10;15) with breakpoints 10p11.1 and 15q11.1. In the literature one case of t(10;15) (q26;q12) in an infertile man with severe ultrastructural sperm alterations is published, indicating diffuse sperm immaturity (Baccetti et al., 2003), but these breakpoints are different from ours.

Another patient was a severe oligozoospermic man (sperm density 0.01 mln/mL, motility 0%) with karyotype 46,XY,t(6;13)(p21.1;q11.2) (*Patient 7*). Non-reciprocal translocation with involvement of acrocentric chromosome 13 may cause severe spermatogenesis disturbances because of its association with the X-Y body. Previously, translocation t(6;13)(p21.2; q33.3) and t(6;14)(q13;q32) have been reported in patients with mental retardation (Bugge et al., 2000). This shows that in clinical manifestation including infertility, the position of breakpoint and genes involved in translocation play a significant role.

One man (*Patient 8*) had severe oligozoospermia (3 mln/mL) and karyotype 46,XY,t(1;15)(p34.1;q26.3)[3]/46,XY[97]. Chromosome 1 has been found to be most frequently involved in rearrangements in infertile males suggesting that chromosome 1 harbours a large chromosomal domain, the integrity of which is important for normal spermatogenesis (Bache et al., 2004). Infertility in our patient may be caused due to both associations between chromosome 1 and X-Y body, and acrocentric (15) and the X-Y body.

We report a karyotype 46,XY,t(7;16)(q21.2;p13.3),inv(9) (p11; q13) in oligoasthenoteratozoospermic man (sperm density 2.6 mln/mL) (*Patient 9*) **(Paper II)** (see 10.1.).

In **Paper IV** we describe a supernumerary inv dup(22)(q11.1) in a severe oligozoospermic man with HH.

His karyotype was: mos 47,XY+inv dup(22)[94]/ 46,XY,t(14;21),+ inv dup(22)[3]/ 48,XY+ inv dup(22)+21[3] in blood (*Patient 10*) (see 10.2.).

In one case (*Patient 11*) there was a karyotype mos 47,XY,+mar[3]/ 46,XY[97] in a man with oligozoospermia (sperm density 13 mln/mL). Because this mar chromosome was found only in 3% of cells, its origin remained unidentified. It is possible that the presence of sSMC even in low percentage mosaic state may cause male infertility.

Sex chromosome abnormalities. They were found in one patient (1.7%), which is similar to the literature data (0.5–4.4%) (Bonaccorsi et al., 1997; van der Ven et al., 1997; Dohle et al., 2002; Rao et al., 2004; Elghezal et al., 2006).

This patient had a mos 47,XXY[92]/46,XY[8] karyotype (*Patient 12*). He was not an azoospermic, but has severe oligozoospermia (sperm count 0.005 mln/mL). This coincides with the literature data that in mosaic 47,XXY individuals a focal spermatogenesis and severe oligozoospermia may be present because of 46,XY cell lines (Visootsak et al., 2006). Mosaic KS has also been found rarely (0.4–0.7%) among oligozoospermics by other authors (De Braekeleer and Dao, 1991; Whitman-Elia et al., 2001; Yoshida et al., 1996). The mosaic 47,XXY males have a risk of producing abnormal gametes with sex chromosome aneuploidy with a frequency in the range of 1.5–7% but in non-mosaic patients it arises up to 45% (Kruse et al., 1998; Lim et al., 1999). So, mosaic 47,XXY males may have a higher chance of having a chromosomally normal foetus in comparison with non-mosaic patients, if artificial reproductive techniques are used.

9.4. Polymorphic chromosomal variants in infertile males

Polymorphic chromosomal variants have been well studied both in the normal population and in infertile males. The incidence of chromosomal variants in normal population varies in different ethnic groups. Previously incidences of Yqh+ and 1qh+ polymorphisms studied in Estonia were not statistically different from our study (Table 19). The frequency of polymorphic chromosomal variants in infertile men was high in our study (37.8%), but similar to that in the control group (43.3%). It was also coincidental with the literature data in infertile males (4.9–58.7%) and in fertile control males (32.6%) (Table 19). Autosomal chromosome variants were more frequent than sex chromosome variants in our study: 35.6% vs. 6.7% in infertile males and 43.3% vs. 3.3% in the control group, respectively.

Heterochromatic polymorphic variants are usually considered as normal variants inherited from one generation to another with low mutation rate and without any direct harmful phenotypic effect due to the scarcity of protein coding regions in them. However, polymorphic variants arisen *de novo* may have some clinical significance and association with clinical anomalies (Madon et al., 2005). The harmful effect of variants may be not direct to phenotype but

indirect through the disturbing normal spermatogenesis and causing the death of germ cells and/or meiotic anomalies resulting in infertility and/or children with congenital anomalies.

Variants of chromosome 1

The incidence of 1qh+ (0.7%) has been previously reported by Tüür et al., (1974) in the normal Estonian population. That is significantly lower ($P<0.05$) than that in infertile men and controls in our study (6.7%). Presumably, it is due to improved diagnose of the variants nowadays, because of growing interest in polymorphic chromosomal variants and consideration of their role in different clinical conditions. A large heterochromatic block in the pericentromeric region of chromosome 1 may affect the pairing of chromosomes causing meiotic arrest, death of germ cells and infertility.

Pericentric inversion of chromosome 1, inv(1) is very rare both in the normal population and in infertile men. We have found it in one infertile man with oligozoospermia (1.1%) and in two men in the control group (6.7%), which is significantly higher ($P<0.05$) than that in the normal Estonian population (0.2%) (Table 19).

Variants of chromosome 9

Incidence of 9qh+ in our study was similar both in infertile males and in the control group (20%). This is higher than that reported in the literature (0.5–14.7%) in infertile males and in the normal population (0–12.9%) (Table 19) (Hamer et al., 1981; Bhasin, 2005). It is suggested that 9qh+ could be in association with repeated spontaneous miscarriages, stillbirth, multiple congenital abnormalities and chromosomal abnormalities in abortus and offspring (see 3.3.). However, the results of our study and many other authors do not support this suggestion because of a very high (20%) frequency of 9qh+ both in normal and infertile males (Bhasin, 2005).

Incidence of pericentric inversion of chromosome 9 was significantly higher in total infertile patients (10%) ($p<0.05$), being in the azoospermia group (15.6%) and in oligozoospermics 6.9%) compared to the control group (3.3%). It was higher than reported in the literature for infertile men (0.7–4.7%) and in the normal population (0–6.3%) (Table 19). Previously, inv(9) has been reported in association with male and female infertility, recurrent miscarriages, congenital abnormalities in offspring and stillbirth (Table 2). It is suggested that inv(9) may often cause infertility in men due to spermatogenic disturbances, which are arisen by the loops or acentric fragments formed in meiosis (Mozdarani et al., 2007). The large block of highly similar sequence can cause genomic instability, via mis-alignment of paralogous segments and unequal crossing-over during meiosis (Horvath et al., 2001). Some interchromosomal effects of inv(9) leading to a higher incidence of mitotic disturbances were also suggested (Madon et al., 2005).

Variants of chromosome 16

We have found variants of chromosome 16 in similar frequency in infertile men (4.4%) and in control (3.3%) that is not significantly different from the literature (Table 19).

Variants of the Y chromosome

We have found sex chromosome polymorphic variants twice as often in infertile men (6.7%) than in control group (3.3%). But this difference was not statistically different ($p>0.05$). The most frequent variant was the long Y chromosome (Yqh+) as has also been shown in the literature data (see Table 19).

Yqh+ was in 5.6% of infertile men (in one man with azoospermia and in 4 men with oligozoospermia) and in 3.3% of the control group in our study. This frequency is similar to that reported in infertile men (up to 7.9%) (Madon et al., 2005). Variation in the frequency of Yqh+ variant between different ethnic groups has been reported to be very large, in some populations up to 40% (in white Australians and in blacks in the US) (Bhasin, 2005). In Estonian males Yqh+ has been previously reported in 4%, which coincides with our data (Mikelsaar et al., 1975) (see 3.3.).

Variants of short-arms of acrocentric chromosomes of D/G groups

Polymorphisms of acrocentric chromosomes of D and G-groups are very rare both in the normal population and in infertile men. This incidence in our study was 2.2%, which was lower than that in the control group (13.3%) ($p>0.05$) being similar to the incidence in infertile males reported by some authors (0.8–2.3%) (Nielsen, 1988; Nakamura et al., 2001; Nagvenkar et al., 2005). However, some authors have reported higher frequencies of acrocentric variants (6.8–25%) (Penna-Videau, 2001; Wiland et al., 2002; Madon et al., 2005; Minocherhomji et al., 2009). Higher frequencies of satellite variants have been found in patients with reproductive failure and spontaneous abortions, and in patients with psychiatric disease. Very large satellites of acrocentrics have been reported in infertile males, but other studies have not shown them as a risk factor of infertility (Penna-Videau et al., 2001; Bhasin, 2005).

Multiple polymorphic variants

We have found more than one variant in 13.3% of infertile males, which half that found in the control group (23.3%) ($P>0.05$). A similar frequency has been reported by Penna-Videau (2001) (13.1%), but it is higher than that reported by others in infertile males (2.3–3.9%) ($P<0.05$) (Wiland et al., 2002; Madon et al., 2005; Nagvenkar et al., 2005). It may be logical to suggest that the presence of some variants together influence more powerfully than a single variant on carriers, and may be associated with male infertility. This problem needs further investigation.

Table 19. Incidence of the most frequent polymorphic variants in infertile males, n(%)

Authors	No. of subjects	1qh+	1qh-	Inv(1)	9qh+	9qh-	Inv(9)	16qh+	16qh-	Yqh+	Yqh-	D/G group	Multi-ple	Total
Madon et al., 2005	458	0	0	0	28 (6.1)	0	3 (0.7)	0	0	36 (7.9)	0	31 (6.8)	18 (3.9)	132 (28.8)
Minocherhomji et al., 2008	380	0	0	0	52 (13.7)	0	4 (1.1)	0	0	102 (26.9)	5 (1.3)	54 (14.2)	–	223 (58.7)
Nagvenkar et al., 2005	88	0	0	0	4 (4.5)	0	1 (1.1)	1 (1.1)	0	4 (4.5)	24 (27.2)	2 (2.3)	2 (2.3)	33 (37.5)
Nakamura et al., 2001	1790	30 (1.7)	0	0	5 (0.5)	0	21 (1.2)	17 (1)	0	0	0	14 (0.8)	–	87 (4.9)
Nielsen, 1988	212	1 (0.5)	0	0	1 (0.5)	1 (0.5)	3 (1.4)	2 (0.9)	0	0	1 (0.5)	4 (1.9)	–	13 (6.1)
Penna-Videau et al., 2001	84	3 (3.6)	0	0	12 (14.3)	0	2 (2.4)	0	0	6 (7.1)	0	21 (25)	11 (13.1)	29 * (34.5)
Wiland et al., 2002	60	0	0	0	3 (5)	3 (5)	3 (5)	0	0	0	0	7 (11.7)	2 (3.3)	15 (25)
Yakin et al., 2005	210	1 (0.5)	0	0	14 (6.7)	0	0	4 (1.9)	0	2 (0.96)	0	0	–	23 (10.95)
Our study Infertile males	**90**	**6 (6.7)**	**1 (1.1)**	**1 (1.1)**	**18 (20)**	**2 (2.2)**	**9 (10)**	**2 (2.2)**	**2 (2.2)**	**5 (5.6)**	**1 (1.1)**	**2 (2.2)**	**12 (13.3)**	**43 * (47.8)**
Control	**30**	**2 (6.7)**	**0**	**2 (6.7)**	**6 (20)**	**3 (10)**	**1 (3.3)**	**0**	**1 (3.3)**	**1 (3.3)**	**1 (3.3)**	**4 (13.3)**	**7 (23.3)**	**13* (43.3)**
Healthy men, Hamer et al., 1981	147	6 (4.1)	0	0	2 (1.4)	0	2 (1.4)	1 (0.7)	1 (0.7)	2 (1.4)	0	7 (4.8)	1 (0.7)	22 (15)
Normal population Bhasin, 2005	73013	(0–8.1)	(0–14.3)	(0–1)	(0–12.9)	(0–24.4)	(0–6.3)	(0–6)	(0.04–23.6)	(0.1–40)	(0.1–11.1)	(4)	–	–
Normal Estonian adults, Mikelsaar et al., 1975	100	–	–	–	–	–	–	–		4 (4)	–	–	–	–
Tüür, 1974	208	(0.7)	–	–	–	–	–	–		–	–	–	–	–

*Some patients had many polymorphic variants simultaneously. (–) No data.

10. Analyses of specific role of genes detected in chromosomal abnormalities in infertile men

10.1. Region of 16p13.3 – loci of protamine PRM1, PRM2 and transition protein TNP2 genes (Paper II)

It is not completely clear by which mechanism reciprocal translocations cause male factor infertility. However, one of the mechanisms may be related to the multitude of genes involved in spermatogenesis. These specific genes could be deleted, or otherwise inactivated by the breakpoints of chromosomal abnormalities (see 3.1.1.). So, it is very important exactly to identify the breakpoints in chromosomes involved in translocations. This allows us to locate the genes to the breakpoint regions, and relate phenotypic characteristics to these genes.

It has been shown that protamine genes (PRM1, PRM2) coding for sperm protamines 1 and 2, and TNP2 gene coding transition protein 2 (TP2) have been involved in spermatogenesis (Domenjoud et al., 1991; Steger et al., 2001). The genes PRM1, PRM2 and TNP2 are located within a 25-kb fragment of chromosome 16p13.3 (Engel et al., 1992). Protamines are small, arginine-rich positively charged, testis-specific nuclear proteins that in the haploid phase of spermatogenesis are required for sperm head condensation and associated transcriptional silencing (Sassone-Corsi, 2002). During normal spermatogenesis, protamines replace the approximately 85% of histones involved in packaging the DNA into the sperm head (Steger, 1999; Aoki et al., 2003). Sperm chromatin is tightly compacted because of the unique associations between the DNA and sperm nuclear proteins, predominantly highly basic protamines. Inter- and intramolecular disulfides between the cysteine residues of protamines are responsible for the compaction and stabilisation of the sperm nucleus. The process of chromatin condensation progresses in two steps: 1) the first step occurs in haploid round spermatids and involves replacement of somatic histones with the transition proteins (TP1 and TP2). 2) Subsequently, in elongated spermatids, the protamines 1 and 2 replace TP1 and TP2. The resulting chromatin is highly condensed and transcriptionally silent (Aoki et al., 2003). As a consequence, the nuclei of mature spermatozoa are very resistant to mechanical and chemical disruption. The nuclear compaction is important to protect the sperm genome from external stresses such as oxidation or temperature elevation (Kosower et al., 1992).

There are many papers about the role of protamines in the male infertility. Protamine gene locus was located on 16p13.3 but no cases of chromosomal abnormality with this breakpoint have been reported (Viguié et al., 1990).

Interestingly, in our patient (patient 9, see 8.3.2.) with translocation between the chromosomes 7 and 16, the breakpoint in chromosome 16 is located in region 16p13.3. It is highly possible that in our patient the breakpoint at 16p13.3 could have disrupted or harboured the PRM1, PRM2 or TNP2 genes coding for sperm protamines 1 and 2, and transition protein 2. Resulting haploinsufficiency of these genes is likely to be the cause of sperm head defects

and infertility in the patient. This case supports the opinion that alterations in the expression of protamine genes may be one of the causes of male factor infertility (Carrell et al., 2001; Belokopytova et al., 1993; de Yebra et al., 1998).

10.2. Region of 22q11.1 (Paper IV)

sSMCs have been reported in association with fertility problems (Shah et al., 2003; Liehr et al., 2004), and have been found in 0.125% in infertile population (compared with 0.044% in live newborns) (Liehr and Weise, 2007).

71 carriers (26 males and 45 females) with supernumerary chromosome 22 (sSMC(22)) with normal phenotype but with infertility problems have been reported (Liehr 2009). From the 26 male carriers of sSMC(22) due to inverted duplication of chromosome 22 of region q10-q11.1 (inv dup(22)(q11.1)) only 3 adult childless males have been published. Two of the males were healthy but their female partners had repeated miscarriages, and one male was infertile with severely depressed spermatogenesis (Manvelyan et al., 2008; Tuerlings et al., 1998). So far, chromosomal abnormalities of the chromosome 22, including inv dup(22)(q11.1) have not been reported in infertile male patients with hypogonadotropic hypogonadism (HH).

We have found (patient 10, see 8.3.2.) the first case of a supernumerary inv dup(22)(q11.1) in infertile male with HH. Our patient was phenotypically normal without clinical features of cat eye syndrome. So, the crossover breakpoint of the supernumerary inv dup(22) in our study occurs proximal to CECR, at the region 22q11.1, between centromere heterochromatin (located 9.6 Mb – 14.4 Mb from 22pter) and CECR, which region begins with 15.6 Mb of 22q as defined by the FISH study reported by Bartsch (2005). This supernumerary inv dup(22)(q11.1) consists of centromeric/pericentromeric heterochromatin but may contain a very small amount of euchromatin in the proximal long arm of chromosome 22. It is reported that sSMCs without euchromatin are harmless and duplications in the near-centromeric region of 22q have been shown to be clinically insignificant (Liehr et al., 2004). But, the literature data showed crossover breakpoints at 22q10–22q11.1 in 26 male patients, whereas in three adult childless males with severely depressed spermatogenesis or with repeated spontaneous abortions in their partners (Tuerling et al., 1998; Manvelyan et al., 2008; Liehr 2009). In addition, there are also 2 unpublished cases of supernumerary inv dup(22)(q11.1) in normal males with infertility problems (cases from Ehresmann and Petersen, from electronic database by Liehr et al., 2004).

The mechanisms, how sSMC leads to male fertility problems are not clear yet. Like any kind of chromosomal abnormalities, supernumerary inv dup(22) may cause incorrect chromosomal pairing during meiosis I, which can cause impaired spermatogenesis and infertility (Shah et al., 2003). It is proposed that male meiosis is more sensitive to the presence of sSMC because it may give the association with the X-Y bivalent at meiotic prophase leading to meiotic arrest

on spermatocyte stage (Oliver-Bonet et al., 2005; Homolka et al., 2007). Another mechanism may be related to the HH, showing hypothalamic or pituitary dysfunction. The molecular basis of HH has been described for only 25-30% of patients, with mutations mostly in the KAL1 (Xp22), FGFR1 (8p12) and GNRHR (4q13) genes. Although there are a lot of HH genes, none of them have been located on chromosome 22. But in our patient with HH there is inv dup(22) with breakpoint 22q11.1, which causes tetrasomy of the centromeric/ pericentromeric region. In the closest vicinity to this breakpoint 22q11.1 (14.4 Mb from 22pter), there have been located segmental duplications or "duplicons" and CNVs structural variations, which often contain gene fragments (Bridgland et al., 2003).

In conclusion. Combining the data from the literature (Tuerlings et al., 1998; Liehr et al., 2009) and our findings, we propose that supernumerary inv dup(22)(q11.1) may be the cause of infertility. The effect on spermatogenesis is probably caused by the association between supernumerary inv dup(22)(q11.1) and X-Y-bivalent. We suggest the first time that either the region 22q11.1 itself or fourfold dosage of centromeric/pericentromeric region sequence may be the cause of HH and infertility in our patient.

11. Other genetic factors (alpha-1 antitrypsin), which influence on the phenotype of infertile patients with chromosomal abnormalities (Paper III)

Klinefelter's syndrome (KS) is a group of chromosomal disorders in which at least one extra X chromosome has been added to a normal male karyotype (Visootsak et al., 2006). The phenotype of patients is variable, but the most constant findings are small and firm testes with hyalinisation of seminiferous tubules, advanced germ cell aplasia, gynaecomastia, hypergonadotropic hypogonadism, azoospermia and infertility, eunuchoid body proportions, increased height, and learning disabilities (Smyth and Bremner 1998; Okada et al., 1999). Additional X chromosome in the karyotype causes lethal gene dosage in the testis environment being responsible for the clinical symptoms including azoospermia and infertility (Johnson, 1998; Thielemans et al., 1998). It is also known that KS patients have frequently other disorders of lung, skin, liver and kidney (Morales et al., 1992; Swerdlow et al., 2005), but this correlation is not well understood yet.

One of the causes of coexisting disorders but also infertility in KS patients may be the low level of alpha-1 antitrypsin caused by mutations in the alpha-1 antitrypsin (ATT) gene (*SERPINE1*). It is highly polymorphic, with more than 100 alleles identified so far. The alleles are categorised into normal, deficient and null variants on the basis of the plasma level and function of AAT. The protein phenotype is classified according to the "Pi" (protease inhibitor) system, as defined by plasma isoelectric focusing. Normal Pi M types account for 95%

of alleles in Caucasian individuals and are characterized by normal plasma levels. Pi X is a rare normal allele variant. Pi Z, S and null types are the most frequent AAT deficiency variants described in lung pathology, but Pi ZZ is associated with both pulmonary and liver diseases (Greene et al., 2008; Kaczor et al., 2007). ATT gene is located on chromosome 14q32.1 (Schroeder et al., 1985).

ATT gene codes alpha-1 antitrypsin, a 52-kDa alpha-1-glycoprotein, which acts as a serine protease inhibitor (serpin) that permeates most body tissues and acts as an inhibitor of a range of proteolytic enzymes. Serpines regulate the activities of a diverse array of serine proteases, controlling complement activation, blood coagulation, inflammation, tumour cell metastasis, and many other physiological processes. Main physiological role of serpin is to inhibit neutrophil elastase and contribute to the innate immune system as an anti-inflammatory protein. It was reported that several serpins play an important role in male reproduction due to contributing to an efficient protease/antiproteinase balance in seminal plasma. A disturbed balance would promote the development of chronic inflammations, which can also be the reason for male infertility problems (Leβig et al., 2010).

Reduced or abnormal production of the AAT protein causes alpha-1 antitypsin deficiency. It is a genetic disorder characterised by low serum level of AAT, and affects about 1 in 3000 of the population in northern Europeans (WHO 1997). AAT deficiency predisposes individuals to the developing severe diseases of lung, liver and other organs. Recent studies reported the relationship between AAT deficiency and male infertility showing that AAT deficiency could favour the deterioration of spermatozoids (Leβig et al., 2010). But there is only one study about AAT deficiency in azoospermic patients with KS in the literature (Varkey and Funahashi 1982).

All our KS patients were azoospermics and infertile with AAT levels in the lower half of the reference value (1.45 g/l) (Table 15). Our finding coincides with the data that azoospermic ejaculates have a lower mean AAT concentration compared to oligo- and normozoospermic ejaculates (Schill 1976). Handelsman et al. found reduced a semen volume in infertile men with homozygous ZZ AAT deficiency (Handelsman et al. 1986). Investigations of a large family have shown that three brothers with Pi FZ phenotype had an unusually small number of offspring when compared to their 8 siblings (1 vs. 39) (Cockcroft et al. 1981). However, there are also contradicting data, which showed that AAT level was significantly higher in 14% of infertile men (Uleova-Gallova et al. 1999). It is known that KS is characterised by additional X chromosome(s), which cause infertility. However, genes for AAT, are not located on chromosome X, but in the region 14q32.1. So, it could be proposed that some genes in X-chromosome interact with 14q32.1 locus where AAT gene is located. It has been suggested that altered expression of AAT in KS (compared to normal ones) may be possible due to differential expression of transcription factors located on chromosome X (Anagnostopoulos et al., 2009).

In conclusion, 2 of 13 KS patients (3 of 24 from combined study with Varkey and Funahashi 1982) had the lowered AAT level and AAT deficiency. Although the group of patients was small and does not allow concrete conclusions to be drawn, our findings show a possibility that the low level of AAT and AAT deficiency may contribute to the risk and pathogenesis of other disorders including azoospermia and infertility, in KS patients.

CONCLUSIONS

1. Major chromosomal abnormalities were found only in infertile men and not in control fertile men. The incidence and type of major chromosomal abnormalities were in correlation with the degree of the impairment of spermatogenesis. Although the total frequency of major chromosomal abnormalities was similar in azoospermic and oligozoospermic men (15.6% and 12.1%, respectively) the types of abnormalities were different. In azoospermic men the main type of chromosomal disorders was 47,XXY (4 cases), and only one patient had chromosomal abnormality of structural type (rob(13;14). Contrary, in oligozoospermic men the main type of chromosomal abnormalities was structural type (6 cases) being mainly translocations between different chromosomes, and only one patient had an abnormality numerical type being mosaic 47,XXY/46,XY. It may be concluded that an additional X chromosome causes more severe impairment of spermatogenesis than structural-type abnormalities between autosomes.

   An important finding was the higher incidence of single cell pathology in infertile men. It may be that in these patients there is a mild form of undetected mosaicism (which is also present in the testes) but the other possibility such as the influence of some harmful environmental factors (medicines) to the chromosomes could not be excluded.
2. Polymorphic chromosomal variants were observed with similar total frequency both in infertile patients (37.8%) and control fertile men (43.3%). However, some polymorphic variants of chromosomes 9 and Y are more frequent in infertile men than in fertile control men. Infertility was observed three times more often in the carriers of inv(9)(p11q13) than in controls. The role of chromosomal variants in male infertility is not fully understood, but it may be that they in some cases (especially with *de novo* origin) disturb normal meiosis.
3. The chromosomal abnormalities found in different disorders offer excellent possibilities to the genotype and phenotype correlation analysis. So we can analyse, which genes in involved chromosomes might be aetiologically correlated to the pathology in patients. We have found the first case of autosomal reciprocal translocation between chromosomes 7 and 16, t(7;16) (q21; p13.3), in which the breakpoint was at the region 16p13.3, where protamine genes have been localised. For the first time by this chromosomal abnormality we could confirm the proposition that alterations in the expression of protamine genes may be one of the causes of male infertility.

   Further, we have shown for the first time that supernumerary inv dup(22)(q11.1) may be a cause of hypogonadotropic hypogonadism either due to the additional chromosome 22q11.1 itself or four-fold dosage of centromeric/pericentromeric region sequence.

4. In addition, there are genes that can modify the clinical features of infertile patients with chromosomal abnormalities. We have found some evidence in favour of hypothesis that the low level and/or deficiency of alpha-1 antitrypsin caused by gene mutations may be one of the factors participating in the pathogenesis of azoospermia and infertility in patients with Kline-felter's syndrome.

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SUMMARY IN ESTONIAN

Meeste infertiilsuse tsütogeneetilised põhjused

Viljatus (infertiilsus) on olukord, kui paaril ühe aasta jooksul kestnud regulaarse suguelu puhul ilma rasestumisvastaseid vahendeid kasutamata, ei ole esinenud rasestumist või ei ole õnnestunud rasedust lõpuni kanda. Infertiilsus esineb 10–15% peredest, olles umbes pooltel paaridel seotud mehepoolse põhjusega. Meeste viljatuse põhjuseid on väga palju, millest 30% moodustavad erinevad geneetilised tegurid eeskätt kromosomaalsed haigused. Kromosoomianomaaliate esinemissagedus on viljatutel meestel kõrge võrreldes üldpopulatsiooniga, ja pöördvõrdeliselt seotud spermatosoidide arvuga. Nii on azoospermiaga meestel (spermatosoidide puudumine seemnevedelikus) kromosoomianomaaliate esinemissagedus eriti kõrge (13,1%–23,6%) võrreldes meestega oligozoospermiaga (spermatosoidide hulk <20 mln/ml) – 2,1–6,6%. Sugukromosoomide arvuanomaaliad esinevad sagedamini azoospermiaga meestel, kuid autosoomide anomaaliad oligozoospermiaga meestel. Autosoomide struktuurianomaaliatest on sagedasemad translokatsioonid, millest enamik on retsiprooksed translokatsioonid. Robertsoni-tüüpi translokatsioone on sagedamini leitud oligozoospermia grupis.

Kõige sagedasem sugukromosoomide arvuanomaalia ja meeste infertiilsuse kromosomaalne põhjus on lisa X-kromosoom(id), mis tekitab karüotüübi 47,XXY ja kliiniliselt Klinefelteri sündroomi (KS), mille kliinilisteks sümptomiteks on hüpogonadism, väikesed testised, seemnejuhade hüalinisatsioon ja fibroos, Leydigi rakkude hüperplaasia, azoospermia ja viljatus. On teada, et KS patsientidel on sageli ka teisi haigusi nagu kopsu-, maksa-, naha-, neeruhaigused, kuid nende seos KS-ga ei ole veel lõpuni selge.

Kromosoomide polümorfismi e. kromosoomivariantide esinemist viljatutel meestel on ka varem kirjeldatud, kuid seos variantide ja spermatogeneesi häirete vahel ei ole veel lõplikult selge. Kromosoomivariantide esinemissagedus on väga varieeruv nii infertiilsetel meestel kui ka üldpopulatsioonis. Kõige sagedasem kromosoomivariant nii infertiilsetel meestel kui ka üldpopulatsioonis on peritsentriline inversioon, inv(9)(p11q13) ja heterokromaatilise ala pikenemine 9. kromosoomil ning Y-kromosoomil. Nende seost reproduktsiooni häiretega on oletatud mõnede autorite poolt.

Kromosoomianomaaliatele lisaks võib terve rida geenidefekte mõjutada fertiilsust. Üle 2000 geeni osaleb erinevates spermatogeneesi etappides ja on seotud meeste arengu ja reproduktsiooni kontrolliga. Geenid, mis on seotud meeste viljatusega, asuvad erinevates kromosoomides, enamasti gonosoomides. Viljatus võib olla geeni defekti ainuke kliiniline ilming muidu fenotüübiliselt normaalsel indiviidil, kuid võib olla ka monogeense haiguse üks sümptomitest.

Eestis on viljatuse tsütogeneetilisi põhjusi seni vähe uuritud. Puuduvad teadmised kromosoomianomaaliate ja kromosoomivariantide seosest meeste viljatusega.

Uurimistöö eesmärgid

Uurimistöö üldiseks eesmärgiks oli hinnata kromosoomianomaaliate seost mehe infertiilsuse tekkes. Uurimuse konkreetseteks ülesanneteks olid:

1) Uurida kromosoomimuutuste olemasolu infertiilsetel meestel ja kontrollgrupis.
   a) Hinnata kromosoomianomaaliate osatähtsust meeste infertiilsuse tekkes
   b) Hinnata heterokromaatiliste polümorfsete kromosoomivariantide osatähtsust meeste fertiilsuses.
2) Uurida detailselt leitud kromosoomianomaaliate olemust ja arutleda nende osa meeste infertiilsuse põhjusena.
3) Analüüsida infertiilsetel meestel leitud kromosoomianomaaliates osalevate geenide spetsiifilist rolli infertiilsuse tekkes: kinnitada seost tuntud geenidega või leida seos uute lookustega.
4) Uurida teisi geneetilisi faktoreid (alfa-1 antitrüpsiin), mis võivad mõjutada fenotüübi kujunemist kromosoomianomaaliatega infertiilsetel patsientidel.

Uuritavad ja meetodid

Tsütogeneetiliselt uuriti 90 lastetut meespatsienti (vanuses 22–42 aastat) TÜ üld- ja molekulaarpatoloogia instituudis inimesegeneetika uurimisgrupis aastatel 1999–2004. Uuritavad saadeti tsütogeneetilisele analüüsile androloogi või uroloogi poolt, kes eelnevalt tegid nende patsientide sperma analüüsi. Sperma analüüside tulemused hinnati vastavalt Ülemaailmse Tervishoiuorganisatsiooni (World Health Organization) kriteeriumitele, mis olid modifitseeritud Andersen jt. poolt. Patsiendid jaotati vähemalt kahe sperma analüüsi alusel kahte gruppi: I grupp (n=32) – azoospermiaga mehed (spermatosoidide puudumine seemnevedelikus) ja II grupp (n=58) – oligozoospermiaga mehed (spermatosoidide hulk <20 mln/ml), kellest 53 patsiendil oli raske oligozoospermia (spermatosoidide hulk <5 mln/ml). Kontrollgrupi moodustasid 30 meest, vanuses 30–45 aastat, kellel oli vähemalt 2 last. Kõik uuritavad allkirjastasid informeeritud nõusolekuvormi, mis oli eelnevalt kinnitatud Tartu Ülikooli Inimuuringute Eetikakomitees.

Kromosoomid uuriti perifeerse vere lümfotsüütide kultuurist Giemsa-vöötide meetodiga. Kromosoomide polümorfismi uuriti CBG-vöötide meetodiga, mis värvib valikuliselt kromosoomide struktuursed heterokromatiini alad. Teisi meetodeid nagu R-, Q-vöödid, AgNOR ja fluorestsents in situ hübridiseerimise (FISH) meetodeid kasutati leitud kromosoomianomaaliate täpsemaks identifitseerimiseks ja analüüsiks.

Klinefelteri sündroomiga patsientidel (13 juhtu) määrati latex-enhanced immunoturbidimeetrilise analüüsiga alfa-1 antitrüpsiini (AAT) tase seerumis ja isoelektrilise fokuseerimisega agaroosi geelil AAT fenotüübid. Kontrolliks kasutati sarnase geneetilise taustaga üldpopulatsiooni andmed (n=1422) (Uibo et al., 1991).

Andmete statistiline analüüs teostati Student'i t-testi, hii-ruut testi ja z-testiga. Kõikide parameetrite statistilist olulisust hinnati tasemel $P<0,05$.

Uurimistöö peamised tulemused ja järeldused

1. Kromosoomianomaaliaid leiti ainult infertiilsetel meestel, kuid mitte kontrollgrupi meestel. Kromosoomianomaaliate esinemissagedus ja tüübid olid seoses spermatogeneesi kahjustuse astmega. Ehkki nende muutuste summaarne sagedus oli sarnane azoospermiaga ja oligozoospermiaga meestel, vastavalt 15,6% ja 12,1%, olid anomaaliate tüübid erinevad. Azoospermiaga meestel oli peamiseks aberratsiooniks lisa X-kromosoom, karüotüüp 47,XXY (4 juhtu), ja ainult ühel patsiendil esines kromosoomide struktuurimuutus – Robertsoni-tüüpi translokatsioon, rob(13;14) (q10;q10). Kuid oligozoospermiaga meestel oli põhiliseks aberratsioonitüübiks struktuurne muutus (translokatsioonid erinevate kromosoomide vahel – 6 juhtu) ja ainult ühel patsiendil esines kromosoomide arvu muutus lisa X-kromosoomi mosaiiksel kujul – karüotüüp mos 47,XXY[92]/ 46,XY[8]. Saadud andmete alusel järeldame, et lisa X-kromosoom põhjustab raskemat spermatogeneesi häiret kui translokatsioonid autosoomide vahel.

   Oluliseks leiuks oli ka ühes rakus esinevate kromosoomiaberratsioonide kõrge esinemissagedus infertiilsetel meestel (eriti azoospermia puhul) võrreldes kontrollgrupiga. See võib olla tõendiks patsientidel esineva varjatud mosaiiksuse kohta (esineb ka testistes), aga ka tõendiks võimalikust keskkonna (ravimite) kahjulikust toimest patsiendi rakkudele.
2. Polümorfsete kromosoomivariantide summaarne esinemissagedus oli infertiilsetel meestel sarnane kontrollgrupiga, vastavalt 37,8% ja 43,3%. Siiski, mõned 9. ja Y kromosoomide variandid esinesid infertiilsetel meestel kontrollgrupist sagedamini. Infertiilsuse puhul leiti, et 9. kromosoomi inversiooni (inv(9)(p11q13)) esines kolm korda kontrollgrupis leitust sagedamini. Ehkki kromosoomivariantide osatähtsus infertiilsuse tekkes ei ole veel täiesti selge, näib siiski, et mõnel juhul võib variandi (eriti de novo tekkega) esinemine põhjustada häireid meioosi normaalses kulgemises.
3. Töö näitas, et kromosoomianomaaliate leidmine patsientidel annab suurepäraseid võimalusi fenotüübi-genotüübi korrelatsioonanalüüsi läbiviimiseks. Saame analüüsida kromosoomianomaaliatest osa võtvates kromosoomides paiknevate geenide spetsiifilist seost patsiendil esineva patoloogia tekkes. Meie leidsime esmakordselt patsiendil retsiprookse translokatsiooni 7. ja 16. kromosoomide vahel, mille puhul murrukoht läbib 16. kromosoomi regiooni 16p13.3, kus paiknevad protamiini geenid. See võimaldas meil esmakordselt kromosoomiaberratsiooni kaudu kinnitada arvamust, et protamiini geenide ekspressiooni muutused võivad olla meeste infertiilsuse tekke põhjuseks.

   Järgnevalt leidsime esmakordselt infertiilsel mehel lisa 22. kromosoomi muutuse – inv dup(22)(q11.1). Leiust järeldasime, et see võib olla üks uus põhjus hüpogonadotroopse hüpogonadismi ja infertiilsuse tekkes tingituna

kas lisa regioonist 22q11.1 või 22. kromosoomi tsentromeerse/ peritsentromeerse regiooni järjestuse 4-kordsest doosist.

4. Lisaks spermatogeneesi mõjutavatele spetsiifilistele geenidele võivad kromosoomianomaaliatega infertiilsete meeste fenotüüpi mõjutada ka teised geenid. Meie leidsime tõendeid hüpoteesile, et alfa-1 antitrüpsiini madal tase või puudulikkus võib olla üheks faktoritest, mis osalevad infertiilsuse ja azoospermia tekkes patsientidel Klinefelteri sündroomiga.

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PUBLICATIONS

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1992–1998	University of Tartu, Faculty of Medicine, MD diploma
1999–2005	University of Tartu, Faculty of Medicine, PhD studies
2010–2011	University of Tartu, Faculty of Medicine, PhD studies, extern

Professional employment

1998–1999	Tartu University Maarjamõisa Hospital, Medical internship
2004–2007	University of Tartu, Institute of General and Molecular Pathology, Department of Human Biology and Genetics, researcher (0.5)
2007–	University of Tartu, Institute of General and Molecular Pathology, Department of Human Biology and Genetics, technician (0.5)
2004–	Finland (different hospitals), physician (0.5)

Scientific work

Main topics of research are related to studies of cytogenetic causes of male infertility.

Co-author of six original publications.

Member of Estonian Medical Association

ELULOOKIRJELDUS

JELENA LISSITSINA

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Haridus

1982–1992	Kohtla-Järve 14. Keskkool, Kohtla-Järve, Eesti
1992–1998	Tartu Ülikool, arstiteaduskond, diplom arstiteaduses
1999–2005	Tartu Ülikool, arstiteaduskond, doktorant arstiteaduses geneetika erialal
2010–2011	Tartu Ülikool, arstiteaduskond, doktorant-ekstern arstiteaduses geneetika erialal

Erialane teenistuskäik

1998–1999	Tartu Ülikooli Maarjamõisa haigla, üldarstlik internatuur
2004–2007	Tartu Ülikool, üld- ja molekulaarpatoloogia instituut, inimese bioloogia ja geneetika õppetool, erakorraline teadur (0,5)
2007–	Tartu Ülikool, üld- ja molekulaarpatoloogia instituut, inimese bioloogia ja geneetika õppetool, laborant (0,5)
2004–	Soome (erinevad haiglad), üldarst (0,5)

Teadustegevus

Teadustöö põhisuunad on olnud seotud meeste viljatuse tsütogeneetilise uurimisega.

Autor või kaasautor kuues teaduspublikatsioonis.

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