CHROMOSOMAL ABNORMALITIES AND POLYMORPHISMS IN THE BACKGROUND OF THE MALE INFERTILITY

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1. Scientific background

Data from the Hungarian Central Statistical Office (KSH) shows that infertility affects about 150,000 couples in Hungary, which means that one in every seven couples has problems in conceiving, and this number increases continuously. The recent examinations showed that in infertile couples, the female and the male factor can be identified equally (40-40%) and in both at 20%. After the andrological or gynaecological examination in the absence of a physiological problem, genetic abnormalities can emerge as the cause of infertility. The genetic examination has been used since 1980 to adjudge the chance of a successful infertility treatment. Nowadays the significance of genetic examination is to diagnose the cause of infertility and to help making conception by assisted reproductive techniques for many couples with severe male factor infertility.

The causes of male factor infertility could be the inadequate volume and quality (pH, fructose content, the time of fluidity) of semen, and the number, motility and morphology of sperms. A marked decline in male reproductive health and an increase in the population of sub-fertile males have been detected worldwide. In the last fifty years the average number of sperm in healthy male reduced by more than 50%. In the case of adequate number and quality of sperms, the problem can be the impairment of sperm transportation. The causes of inadequate sperm production, maturation or transportation could be congenital disorders, or could develop later, in consequence of varicocele, infection of the genital and excretory system, endocrine, immunological, or obstructional problems, chronic illnesses, tumours or its treatments, lifestyle or genetic aberrations (chromosomal disorders, polymorphisms and mutation of genes).

2. The objective of the dissertation

The aim of our examination was to investigate the genetic background of male infertility with the following sub-tasks:
— Investigation of chromosome aberrations in infertile male
— Comparison of normal chromosome polymorphisms and its incidence in infertile and fertile control men to examine the connection between chromosome polymorphisms and decreased fertility or infertility.
— Examination of interchromosomal effects: incidence of chromosomal polymorphism collectively and with chromosomal aberrations.
— Examination of the possible connection between the phenotype and the chromosomal abnormalities and/or polymorphisms.

3. Subjects and methods

The examined male subjects were selected from mainly the Andrological Clinic of Semmelweis University, and other hospitals, health centre or private doctors with diagnosis of infertility during 2003 and 2006. Those men were included in the examined group from the 2790 infertility male applied for andrological examination who were suitable for the professional protocol of the Ministry of Health for the genetic examination of reproductive failures (Klinikai Genetikai Szakmai Kollégium 2006). According to the protocol those males were taken into the examination who were azoospermic or severe oligospermic and there was no physical explanation to their infertility and whose wife had an abortion several times. Sixty of these men applied for assisted reproduction technique and undertook the genetic examination.

Routine andrological investigation included physical examination, 2 semen analyses, ultrasound of the testes, and assessment of hormone levels. The cytogenetic examination was made at the Human Genetic Laboratory, Institute of Forensic Medicine, Semmelweis University. The used chromosomal staining methods were the following: GTG, QFQ, CBG and NOR banding techniques. The molecular cytogenesis examination was made at the Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences. The molecular genetic examination of Y chromosome
was prepared in the Medical Laboratory, Würzburg and in the Laborigo Molecular Genetics Laboratory, Budapest.

The infertile male group was compared with 568 fertile male (control group) who took part in paternity cases in our laboratory.

In the statistical evaluation of our results, we analysed (with \( \chi^2 \) probe) the absolute and relative incidence of chromosomal abnormalities and polymorphisms compared with control group.

4. Scientific results

We summarize the results of this dissertation in the followings:

Thesis 1: *Chromosome abnormalities in infertile men*

Our results confirmed that the incidence of male infertility caused by chromosomal disorders is similar to the same data in the European population.

— The Klinefelter syndrome (8.3%) was the most frequent disorder in our study. According to the literature the incidence of this syndrome is 3-11%.

— In the infertile group we found high incidence (3.3%) of extra Y chromosome, and Y chromosome microdeletion.

The men with 47,XYY syndrome are usually fertile according to literature data. The incidence of this syndrome is 0.1% in the common population. In the case of our examined group the cause of the bad results of spermiogram (azoospermia and severe oligospermia) could be other harmful effects.

The results of our examination about Y chromosome microdeletion confirmed that the male or female phenotype is caused by the presence or absence of the SRY gene, not the proportion of the mosaic cells. The occurrence of short stature can be accounted by the deletion of the GCY loci or the haploinsufficiency of the SHOX gene. The failure of
spermatogenesis can be explained with the deletion of a major part of the AZF region or the high proportion of the 45,X cells or of these effects taken together.

— In our infertile group we did not detect autosomal aberrations in the background of infertility, but in the control group the incidence of translocation was 0.7%, and the found t(22;Y), t(21;22), t(13;14), t(2;13) translocations did not cause infertility in these men. On the basis of the above mentioned, we can state, in contrast to literature, that these aberrations do not cause infertility alone, but only together with other factors. Moreover, the assertion that infertility was caused by this structural abnormality because of the deletion of some genes, needs the exact molecular analysis of the aberrations and knowledge of the breakpoint.

Altogether the incidence of chromosomal abnormalities (15.0%) is much greater in the infertile male group than the control group (0.4%). According to literature the incidence of chromosomal abnormalities in common population (0.38%) is similar to our results. In the case of infertile men the incidence of chromosomal abnormalities depends on the decrease of the fertility: 2% in men with decreased fertility, 5% in oligozoospermic men, and 10-15% in azoospermic men. Our examined infertile group is at the upper limit of the literature data because of the vast majority of azoospermic men. The types of chromosome disorders were also different in the two groups, sex chromosome disorders were found in infertile men and autosomal disorders in fertile men.

Thesis 2: Comparative analysis of chromosome polymorphisms in fertile and infertile men

— Incidence of extreme size (very large and very small) polymorphisms of Y chromosome was higher than the expected value; hence the Y chromosome with extreme size can be in connection with infertility.

— Examination of the incidence of fluorescence polymorphisms of Y chromosome did not show significant difference between the fertile and infertile men.

— We found significant differences between the fertile and infertile men in the distribution of heterochromatin size polymorphisms of chromosome 9.
— The total pericentric inversion of chromosome 9 was more frequent in the infertile group than the control group. Those men that have total pericentric inversion in chromosome 9 have a higher probability of infertility because the inversion modifies the morphology, motility and meiotic segregation of sperms.

— Examination of the fluorescence polymorphisms of chromosome 3, 4 and acrocentrics showed that both examined parts (near the centromere and satellite) of the chromosome 13, 14 and 21 had significant differences between the fertile and infertile men.

— The number of satellites differed from the normal number of two more frequently in the infertile group than in the control group. The numeral polymorphisms of satellites were the most frequent variants in chromosome 15. The number and size of the satellites affects the infertility by the association ability of the acrocentric chromosomes, which increases the possibility of Robertsonian translocation because of the greater fragility of these chromosomes, and cause aneuploidy by impair meiotic segregation.

Thesis 3: Examination of interchromosomal effects

The extreme size (very large and very small) of heterochromatin of chromosome 1 and 9, and the total pericentric inversion of chromosome 9 were more frequent in men with chromosomal aberration (sex chromosomal aneuploidy) than the whole infertile male group. The extreme size and position polymorphisms of the heterochromatin of chromosome 9 could increase the frequency of chromosomal aberrations (mainly the aneuploidies) through interchromosomal effect impairing the cell division.
Thesis 4: *Examination of the phenotype in the case of chromosomal abnormalities and polymorphisms*

— The various phenotypes of men with Klinefelter syndrome could be explained with additional effects of other genes. According to our results the autosomal heterochromatin polymorphisms may also modify the phenotypical appearance of men with Klinefelter syndrome. Extreme polymorphisms of chromosome Y and short arms of acrocentric chromosomes do not affect the phenotype of these men.

— The bad results of spermatogram of men with 47, XYY syndrome could be caused by varicocele and other genetic effects such as the position polymorphisms of heterochromatin of chromosome 9.

— In case of men with microdeleted Y chromosome the degree of decreased fertility is determined by the size of deletion, e.g. the number and function of the deleted genes. In addition to these, damage of genes in the Y chromosome can cause other malformation of the phenotype, such as short stature by deletion of the GCY gene controlling growth or the haploinsufficiency of SHOX gene.

— After examining the connection between the chromosome polymorphisms and phenotype in the whole infertile group, we have concluded the following results:

  - Obesity, testicular malformations and gynecomastia were not more frequent events in men with size and fluorescence polymorphisms of Y chromosome than in the whole infertile male group.

  - Malformation of all examined phenotypic features was significantly more frequent in men with extreme size polymorphisms and/or total inversion of autosomal heterochromatins than in the whole infertile male group.

  - Malformation of all examined phenotypic feature were significantly less frequent in men with extreme numerical and fluorescence polymorphisms of satellites than in the whole infertile male group.
5. Conclusion

In addition to the routine andrological examination of infertile men, the necessity of genetic investigations can be proved by the numerous chromosome disorders. With the precise diagnosis of a disorder we could save these patients from long and expensive examinations and unsuccessful procedures. After the well drew up anamnesis and extensive examination with the knowledge of the exact diagnosis, we can find the adequate medical attendance. In the case of men with microdeleted Y chromosome not only the causes of infertility can be found by the diagnosis of the disorder, but also the probable results of the testicular biopsy can be predicted with the knowledge of the location and extension of the deletion. In the case of a confirmed microdeletion, cryopreservation of the sperm at youth is advised since the number of sperm dramatically decreases with age. We should pay special attention to these patients also because of the transition of the disorder to the next generation, where the deletion can affect greater part of the Y chromosome than in the father. Moreover, in the case of a male with sex chromosome anomaly, we need to consider a higher risk of fathering a child with autosomal or gonosomal aneuploidies after a successful fertilisation treatment.

In addition to the human rights to health and recovery, the acceptance of the right of having a baby should be the basis of the modern society. At the same time achieving conception of infertile couples generate elementary ethical problems. We have to take into account the consequences of infertility treatment for the population, because genetic contra selection is a great expense to the genetic matter of the population. It means that besides the low mean number of children and the common descending number of sperms, the sub-fertile men taking part in reproduction more frequent by fathering a child with persistent endeavour or assisted reproductive techniques. Transmitting their genetic weakness to their offspring the sub-fertile or infertile men decrease the reproductive ability of the next generation in a statistical sense.
6. Publications

1. Directly related to theses:

2. Other publications:

3. Conference books and abstracts:


4. Professional representations, educations: