

Genetic Causes of Infertility: Chromosomal Abnormalities in Couples with Reproductive Failure

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Abstract

Objectives. This study analyses the prevalence of karyotype changes and Yq11 micro-deletions among infertile couples referred for assisted reproduction techniques.

Methods. Each partner of 517 infertile couples referred Life Memorial Hospital was screened for karyotype changes by GTG-banding technique on peripheral lymphocytes. No subject presented with obvious phenotype of chromosomal rearrangement. All the oligo/azoospermic men with normal karyotype were further investigated by PCR for Yq11 micro-deletions.

Results. 11 out of 517 couples (2.12%) had one partner carrying a chromosomal change, 1 out of 24 (4.16%) men showed Yq11 micro-

deletions and 1 showed SRY translocation on the short arm of the chromosome X (4.16%). The chromosomal rearrangements were 11 (1.06%) translocations, 3 (0.29%) 47,XXY, 1 (0.09%) 45,X0, 2 (0.19%) marker chromosomes, 2 (0.19%) inversions.

Conclusions. Genetic testing and genetic counseling in infertile couples attending assisted reproduction are important to recognize the cause of infertility, but also to evaluate the reproductive risk of couples with genetic abnormalities that may be transmitted to offspring.

Keywords: Infertility, genetic, chromosome, karyotype

Background

Reproductive endocrinologists consider a couple to be infertile if:

- the couple has not conceived after 12 months of contraceptive-free intercourse if the female is under the age of 34;

- the couple has not conceived after 6 months of contraceptive-free intercourse if the female is over the age of 35 (declining egg quality of females over the age of 35 account for the age-based discrepancy as when to seek medical intervention);

- the female is incapable of carrying a pregnancy to term.

Prevalence

Generally, it is estimated worldwide that one in every seven couples has problems conceiving, with the incidence similar in most countries independent of the level of the country's development. Most couples (about 84 out of every 100) who have regular sexual intercourse (that is, every 2 to 3 days) and who do not use contraception will get pregnant

within a year. About 92 out of 100 couples who are trying to get pregnant do so within 2 years.

Women become less fertile as they get older. For women aged 35, about 94 out of every 100 who have regular unprotected sexual intercourse will get pregnant after 3 years of trying. For women aged 38 however, only 77 out of every 100 can still do so. The effect of age upon men's fertility is less clear.

Factors that can cause male as well as female infertility are:

- Genetic Factors
- General factors - Diabetes mellitus, thyroid disorders, adrenal disease
- Hypothalamic-pituitary factors - Kallmann syndrome, Hyperprolactinemia, Hypopituitarism
- Environmental Factors - Toxins such as glues, volatile organic solvents or silicones, physical agents, chemical dusts, and pesticides.

For some couples attempting pregnancy, something goes wrong in this complex process, resulting in infertility. The cause or causes of infertility can involve one or both partners.

Causes of Male Infertility

A number of things can cause impaired sperm count or mobility, or impaired ability to fertilize the egg. The most common causes of male infertility include abnormal sperm production or function, impaired delivery of sperm, general health and lifestyle issues, and overexposure to certain environmental elements.

Impaired production or function of sperm. Most cases of male infertility are due to problems with the sperm, such as:

- **Impaired shape and movement of sperm**
- **Low sperm concentration**
- **Variococele**
- **Undescended testicle**
- **Testosterone deficiency** (male hypogonadism)
- **Genetic defects.** In the genetic defect Klinefelter's syndrome, a man has two X chromosomes and one Y chromosome instead of one X and one Y. This causes abnormal development of the testicles, resulting in low or absent sperm production and possibly low testosterone.
- **Infections.** Infection may temporarily affect sperm motility. Repeated bouts of sexually transmitted diseases (STDs), such as Chlamydia and gonorrhea, are most often associated with male infertility.

In many instances, no cause for reduced sperm production is found. When sperm concentration is less than 5 million per milliliter of semen, genetic causes could be involved. A blood test can reveal whether there are subtle changes in the Y chromosome.

Impaired delivery of sperm. Problems with the delivery of sperm from the penis into the vagina can result in infertility. These may include:

- **Sexual issues**
- **Retrograde ejaculation**
- **Blockage of epididymis or ejaculatory ducts**
- **No semen (ejaculate).** The absence of ejaculate may occur in men with spinal cord injuries or diseases.
- **Misplaced urinary opening** (hypospadias)
- **Anti-sperm antibodies**
- **Cystic fibrosis.** Men with cystic fibrosis often have missing or obstructed vas deferens.

General health and lifestyle. A man's general health and lifestyle may affect fertility. Some common causes of infertility related to health and lifestyle include:

- **Emotional stress**
- **Malnutrition.** Deficiencies in nutrients such as vitamin C, selenium, zinc and folate.
- **Obesity.** Increased body mass.
- **Cancer and its treatment**
- **Alcohol and drugs**
- **Other medical conditions.** Certain diseases or conditions, such as diabetes, thyroid disease, Cushing's syndrome, or anemia may be associated with infertility.
- **Age.** A gradual decline in fertility is common in men older than 35.

Environmental exposure. Overexposure to certain environmental elements such as heat, toxins and chemicals can reduce sperm count either directly by affecting testicular function or indirectly by altering the male hormonal system. Specific causes include:

- **Pesticides and other chemicals**
- **Overheating the testicles**
- **Substance abuse** (use of cocaine or marijuana may temporarily reduce the number and quality of your sperm)
- **Tobacco smoking.**

Causes of Female Infertility

The most common causes of female infertility include fallopian tube damage or blockage, endometriosis, ovulation disorders, elevated prolactin, polycystic ovary syndrome (PCOS), early menopause, benign uterine fibroids and pelvic adhesions.

■ **Fallopian tube damage or blockage.** Fallopian tube damage usually results from inflammation of the fallopian tube (salpingitis).

- **Endometriosis**
- **Ovulation disorders**
- **Elevated prolactin** (hyperprolactinemia)
- **Polycystic ovary syndrome** (PCOS)
- **Early menopause** (premature ovarian failure)
- **Benign uterine fibroids**
- **Pelvic adhesions.**

Other causes: A number of other causes can lead to infertility in women:

- **Medications**
- **Thyroid problems**
- **Cancer and its treatment**
- **Other medical conditions.** Cushing's disease, sickle cell disease, HIV/AIDS, kidney disease and diabetes, can affect a woman's fertility.
- **Caffeine intake.**

Genetic causes of infertility

Problems in the genes that regulate male fertility and in the genetic material of sperm itself are important contributors to infertility problems in men. In fact, even in men with no known fertility problems, 19% of the sperm are genetically defective^[1]. Certain inherited medical conditions also contribute to male infertility. Defective genes themselves can be inherited, produced by environmental assaults (such radiation exposure), or both. Of some concern is the possibility that these mutations will be passed to offspring in men who undergo fertilization techniques that retrieve sperm and directly fertilize the egg. (Under natural conditions, genetically abnormal sperm would be very unlikely to reach and fertilize the egg.)

Defective Genetic Material. Sperm carry half the genetic material necessary to make a human being. Infertile men have been reported to have a relatively high percentage of sperm with broken or damaged DNA (the molecular chain that makes up a gene)^[1].

Genetic Factors Specifically Affecting Sperm Production or Quality. Abnormalities in genes that specifically regulate sperm production and quality are major factors in male infertility. Some research suggests that about 10% of cases of male infertility may be due to problems, most likely genetic, in the

acrosome. The acrosome is the enzyme-filled membrane cap on the sperm - its warhead - that is critical for piercing the egg. In one study, pregnancy was impaired if 7% or more of sperm had abnormalities in the acrosome^[2].

Inherited Disorders that Affect Fertility. Certain inherited disorders can impair fertility. Examples include:

■ Cystic fibrosis patients often have missing or obstructed vas deferens (the tubes that carry sperm). In fact, men whose infertility is caused by an inborn missing vas deferens have a 60% chance that they carry the gene for cystic fibrosis (even if they don't have the disease itself)^{[3],[4],[5]}. Some genetic defects, instead of involving an entire chromosome, affect a single gene. An example of this is the most common mutation seen in the Caucasian population, a mutation in the gene that causes the disease Cystic Fibrosis. As it turns out, men who are unaffected with CF, but carry a single copy of the gene (a "carrier" for Cystic Fibrosis), are at high risk to have a condition called "congenital absence of the vas deferens". Men with this condition do not have sperm in the ejaculate, but have abundant sperm in the epididymus and testicle, which is retrievable for IVF and ICSI. This is an example of a single-gene mutation that results in a specific type of male infertility. All men with congenital absence of the vas deferens should be tested for CF gene mutations. Their female partners need to be tested in order to know whether or not the couple will be at risk to have a child with the disease.

■ Kartagener syndrome, a rare disorder that is associated with a reversed position of the major organs, also includes immotile cilia (hair-like cells in lungs and sinuses that have a structure similar to the tails of sperm). Sperm motility may also be impaired by this condition.

■ Polycystic kidney disease, a relatively common genetic disorder that causes large cysts to form on the kidneys and other organs during adulthood, may cause infertility as the first symptom if cysts develop in the reproductive tract.

■ Other genetic causes - Abnormalities in the testosterone (androgen) receptor gene. This is a very rare condition in which the tissues of the body cannot

recognise and respond to testosterone in the blood.

■ Y Chromosome deletions (missing genetic information on the Y chromosome)

In most cases, the cause of severe male factor infertility is unknown. However, recent work from the laboratories of several scientists have reported that about 10-15% of men with severe male factor infertility (less than 2 million sperm per cc), when specifically tested, will be found to have a small portion of genetic material (DNA) missing from the Y chromosome^[6]. This is a particular type of single-gene mutation that is associated with low sperm counts or lack of any sperm in the ejaculate. It does not appear that this deletion is associated with any abnormality other than male infertility. Many men have been able to conceive with this condition through IVF with ICSI. A recent scientific presentation reported that of ten babies born to fathers with known partial Y chromosome deletions, all the male babies studied had the same deletion as their fathers. That is, they will also be likely to be infertile. Although preliminary, it does not appear that this deletion is worse in the children. That is, there is no finding of larger pieces of missing DNA in the sons, therefore, no evidence that the abnormality is getting worse with a subsequent generation.

The Y chromosome is not essential for life and until recently most regions of it were assumed to be functionally inert. Sex determination (controlled by the SRY gene) has long been viewed as the sole function related to the Y chromosome, but this theory changed in recent years when another important function (the control of spermatogenesis) was discovered and many genes were mapped to the Y chromosome. Spermatogenesis is a long and complex process requiring about 70 days and involving an elaborate succession of distinct cell types generated by mitotic and meiotic divisions. In the initial stages, spermatogonia divide via mitoses, giving rise to primary spermatocytes, which in turn undergo the first meiotic division leading to secondary spermatocytes. Through the second meiosis these cells produce haploid cells (round spermids), which elongate during the spermiogenesis process (elongated sper-

matids) and finally differentiate into mature spermatozoa, by condensation of the chromatin, substitution of histones with protamines, and formation of the acrosome and the other sperm components. However, our knowledge of the mechanisms regulating spermatogenesis is still poor, and only recently has research focused on the identification of genes specifically involved in its regulation. Nevertheless, infertility is a major health problem affecting 10-15% of couples seeking to have children, and a male factor can be identified in about half of these cases. A significant proportion of infertile males are affected either by oligozoospermia (reduced sperm production) or azoospermia (lack of any sperm in the ejaculate). Such alterations in sperm production may be related, in turn, to different underlying testicular histological pathologies, ranging from the complete absence of germ cells (Sertoli cell-only syndrome) to hypospermatogenesis and maturation arrest. The alteration of spermatogenesis can be the consequence of many causes, such as systemic diseases, cryptorchidism, endocrinological disorders, obstruction/absence of seminal pathways, or infections. However, the cause of male infertility is unknown in up to 50% of cases, and until recently relatively little research focused on the possible genetic etiologies. The explosive growth of assisted reproduction techniques and, in particular, of intracytoplasmic sperm injection (ICSI) has contributed to the development of such research^[7].

■ Klinefelter syndrome patients carry two X and one Y chromosomes (the norm is one X and one Y), which leads to the destruction of the lining of the seminiferous tubules in the testicles during puberty, although most other male physical attributes are unimpaired.

■ Other Chromosome Abnormalities.

It is possible that there is a wider association between genetic abnormalities and infertility in both men and women. Several studies have been published in which chromosome analysis (karyotyping) has been performed on both men and women entering IVF treatment. These studies have reported that the incidence of the male and female partners with infertility

problems have about a 4-9% chance themselves of carrying a chromosomal abnormality, including sex chromosome abnormalities^[6]. This incidence reported in mostly small studies appears to be higher than the overall incidence of chromosomal abnormalities in the general population, which is about 0.7% of all live births. It is unclear if these abnormalities are the cause of the infertility. Considering the types of some of the abnormalities reported, there is clearly the potential for some of these to increase the risks of conceiving an abnormal embryo that will not be able to establish a normal pregnancy. The cost of performing a karyotype analysis is probably not routinely covered under insurance benefits for an infertility evaluation.

Constitutional aberrant karyotypes can account for infertility or recurrent pregnancy loss. When present in the germinal lineage, chromosomal abnormalities can be segregated in gametes and transmitted to the offspring, while in other cases they can hamper meiosis up to the arrest of gametogenesis, or may give rise to unbalanced gametes^{[8],[9]}. In mammals, gametes carrying chromosomal aberrations have a poor chance of successfully undergoing fertilization.

Natural selection of cells with integer genome usually prevents the formation of zygotes with major abnormalities.

The present study offers our contribution on the topic by a retrospective analysis of the prevalence of chromosomal abnormalities in a population of 517 infertile Romanian couples.

Methods

Clinical files of 517 infertile couples, referred to Life Memorial Hospital for reproductive failure were reviewed in this

study. All cases were white Caucasians. Investigation for chromosomal anomalies was routinely performed by cytogenetic analysis of both partners of each couple. The karyotypes were performed by GTG-banding technique studying metaphases of proliferating lymphocytes from peripheral blood. Structural and numerical autosomal aberrations were defined on the basis of at least three metaphases.

Chromosome polymorphisms such as changes in size of heterochromatin regions and pericentric inversions of chromosome 9 were not considered. All the men affected by severe oligozoospermia or azoospermia had a normal karyotype were further investigated for the presence of micro-deletions of the three AZF loci in Yq11 (AZFa, AZFb and AZFc).

Azoospermia, severe oligozoospermia and mild oligozoospermia were defined as the total absence of sperm cells in seminal liquid, a sperm cell count <5 x 10⁶ cells/ml, and a sperm cell count >5 x10⁶ and < 20 x 10⁶ cells/ml respectively. Each diagnosis of oligo/azoospermia was achieved by at least two consecutive spermograms performed in ejaculated semen after centrifugation of the specimens.

The search for micro-deletions was carried out by amplifying, with PCR, 10 sequence-tagged sites (STS) encompassing the AZFa, AZFb and AZFc loci.

Results

A total of 211 couples out of 517 studied (40.80%) never achieved pregnancy, namely each female partner had history of gravidity 0 and parity 0 (G0P0 group); the remaining

306 (59.18%) had history of at least one spontaneous pregnancy loss within

the 12th gestation week, (G>1P0 group).

Observed numerical chromosome aberrations were complete 47, XXY karyotype in 3 cases, 47 XXX in 1 case and 45 X0 in 1 case, presence of a marker chromosome in 2 cases. The complete gonosomal aneuploidies were found in the male group, namely the 2 cases of 47,XXY, which configures the Klinefelter syndrome. All these subjects were totally azoospermic. Marker chromosomes were found in one male, and one female. No chromosomal anomalies were detected in five patients with obstructive azoospermia. Structural chromosome changes observed in the whole cohort were 3 autosomal reciprocal balanced translocations, 7 Robertsonian translocations, one inversion. All these anomalies were observed in 100% of metaphases. The prevalence of whole translocations in men was 1.35% and in women was 0.77%. Prevalence of karyotype changes among women with various gravidity histories shows some differences. In the group of female partners the prevalence of chromosomal aberrations was higher among the women with G1P0 history in comparison to those with G0P0 history, i.e. 0.96% of the G 1P0 women compared to 0.58% of the G0P0 women. In the male group the prevalence of karyotype changes among the partners of either G 1P0 or G0P0 couples also shows some differences, i.e. 1.16% of men belonging to G1P0 couples in comparison to 1,54% of the men of the G0P0 couples.

In all, 24 out of 517 male subjects (4.64%), all with normal karyotype and belonging to the G0P0 group, were also investigated for Yq11 micro-deletions due to poor semen quality spanning from severe oligozoospermia to total azoospermia.

One of these patients (4.16%) showed a Yq11 microdeletion, involving the AZFc locus.

In one male subject belonging to the G0P0 group, we found a 46, XX karyotype. In this case FISH analysis revealed a SRY signal on the short arm of the chromosome X (XX male syndrome).

Discussion

In this prevalence study, only infertile couples were analyzed. As is usual in several European infertility centers, both partners of each couple underwent

Table 1

General features of the studied population

No. of couples	517
Age of women (years)	33.08
Age of men (years)	36.64
Duration of infertility (years)	3.82
Couples with history of:	
gravidity 1, parity 0	306 (59.18%)
gravidity 0, parity 0	211 (40.8%)

Table 2

Karyotype findings among the male partners of the couples with history of gravidity >1 and parity 0 (306 couples)

Karyotype	Frequency
45,XY t(13;14)(p11;q11)	3 (0.98%)
45,XY t(13;15) (p11;q11)	1 (0.32%)
45,XY t(21;21) (p11;q11)	1 (0.32%)
47,XY + mar	1 (0.32%)

Table 3

Karyotype findings among the male partners of the couples with history of gravidity 0 and parity 0 (211 couples)

Karyotype	Frequency
47,XXY	3 (1.42%)
46,X del(Y)(q11)	1 (0.47%)
46,XY t(3;13)(p21;p11)	1 (0.47%)
46,XY t(1;19)(p13;p13)	1 (0.47%)
46,XX, SRY positive	1 (0.47%)
46,XY inv(1)(p13;q23)	1 (0.47%)

Table 4

Karyotype findings among the female partners of the couples with history of gravidity 1 and parity 0 (306 couples)

Karyotype	Frequency
47,XXX	1 (0.32%)
45,XX t(13;14)(p11;q11)	1 (0.32%)
45,XX t(14;15)(p11;q11)	1 (0.32%)
46,XX inv(8)(p22;q13)	1 (0.32%)
47,XX + mar	1 (0.32%)

Table 5

Karyotype findings among the female partners of the couples with history of gravidity 0 and parity 0 (211 couples)

Karyotype	Frequency
45,X0	1 (0.47%)
46,XX t(10;19)(q22;q13)	1 (0.47%)
45,XX t(13;14)(p11;q11)	1 (0.47%)

karyotyping before entering assisted reproduction treatment. This practice is aimed at reducing the incidence of pregnancy losses or congenital anomalies owing to genetically unbalanced gametes originated by parents carrying some chromosomal rearrangement. Moreover in this study we chose to report all the chromosomal abnormalities which might have some clinical expression, including those whose role in the pathogenesis of infertility is not clear, in order to add another baseline to further studies approaching the same problem. Infertile couples have a higher risk of constitutional chromosome rearrangements which may be the cause of infertility^{[8],[9],[10],[11]}, it is important to exclude the most common genetic causes of infertility in the couple before they undergo medically assisted reproduction because genetic abnormalities may be inherited by offspring. Many authors have underlined that in a selected population, such as couples undergoing ICSI, the prevalence of karyotype aberrations and Yq micro-deletions is higher than in the general population^{[8],[10]}. In males the prevalence of genetic factors seems to be inversely related to the sperm count. The incidence of chromosome factors in infertile males ranges between 2 and 8%, increasing to 15% in azoospermic males^[12]. Structural and numerical chromosomal abnormalities may interfere with the gametogenic process and cause infertility in both sexes by leading to the production of unbalanced gametes or the arrest of gametogenesis^{[2],[10],[14],[13]}.

In the work-up of severe male infertility with non-obstructive azoospermia and severe oligozoospermia, the screening of micro-deletions of the AZF region of the Y chromosome is also recommended. Our data, referred to 517 infertile couples showed an increased prevalence of chromosomal abnormalities in both sexes compared to the general population (i.e., couples who have spontaneous conception). Chromosome aberrations between the two sexes in our study group showed some differences: 14 cases out of 517 men (2.70%) and 8 cases out of 517 women (1.54%). In both male and female groups, the structural alterations of the autosomes were more frequent.

The frequency of the sex chromosome aneuploidies (0.48%) is lower than that found in the literature (1.8%)^[10].

The prevalence of autosomal structural aberrations in our female group (0.96%) was lower than that found in literature 2.08-2.1%^{[8],[12]} we found 2 reciprocal and 2 Robertsonian translocations and one chromosome 8 inversion.

The incidence of chromosomal abnormalities found in our study in the female group (1.54%) was lower than the data reported in the literature (5%)^[12]. In the male group we found 1.93% autosomal structural aberrations. Among these the most frequent was the Robertsonian translocation (0.96%) referred to in the literature and associated with infertility by spermatogenesis impairment^{[15],[13]} and with miscarriages^{[13],[16]}.

Furthermore, reciprocal translocations, found in 0.38% of our patients, are associated with infertility^[11]. Only 1 de novo inversion of chromosome 1 was found which is also associated with infertility, and especially with azoospermia^[17].

We did find 3 cases of Klinefelter karyotype (47,XXY) that has been reported as the most frequent chromosome aberration causing azoospermia in men undergoing ICSI.

The prevalence of Klinefelter syndrome among infertile men is very high, up to 5% in severe oligozoospermia and 10% in azoospermia^{[6],[10],[18]}.

Concerning numerical aberrations, we found two supernumerary marker chromosomes (ESACs) in patients. The frequency of ESACs in our study was similar (0.19%) to the general population (0.14-0.72‰). An increased frequency of autosome numerical aberrations, including ESACs, in the infertile men has also been reported^{[19],[11]}.

In the work-up of severe male infertility with non-obstructive azoospermia and severe oligozoospermia, the screening of micro-deletions of the AZF region of the Y chromosome is also recommended. In the AZF region of the Y chromosome 3

different spermatogenesis loci (AZFa, b, c-d) have been mapped. Micro-deletions in these regions cause severe testiculopathy and infertility in 2% of male infertility^{[18],[19],[20]}. The prevalence of Yq micro-deletions increases to 15-20% in selected males affected by severe oligozoospermia or non-obstructive azoospermia^{[12],[23],[24]}.

Conclusions

Our results indicate, in line with other authors, that karyotype analysis in both partners and Yq micro-deletion analysis in male partners with a sperm count of 5 x 10⁶/ml should be performed for infertile couples.

Genetic testing and genetic counseling in infertile couples attending assisted reproduction are important to recognize the cause of infertility, but also to evaluate the reproductive risk of couples with genetic abnormalities that may be transmitted to the offspring. ■

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