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Original Research

Chromosomal Abnormalities in Infertile Greek Men: A Single Institution's Experience

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Abstract

Chromosomal abnormalities represent a significant genetic cause of male infertility because they impair spermatogenesis. The objective of the current study was to determine the prevalence and distribution of chromosomal abnormalities in Greek men with infertility. Four hundred eighty-eight infertile men (27 azoospermic, 168 with oligospermia -98 mild, 57 moderate, 13 severe- and 293 with normospermia) undergoing In *Vitro* Fertilization (IVF) between 2016-2022 were enrolled in the study. Thirty-eight fertile men were also studied. Chromosomal analysis of peripheral blood lymphocytes was performed using standard cytogenetic techniques. 21/488 (4.3%) of men tested had an abnormal karyotype; 13 (2.7%) had sex chromosome abnormalities and 8 (1.6%) had autosomal ones. No chromosomal aberration was detected in the control group. The frequency of chromosomal alterations was significantly higher in azoospermic men than in men with oligospermia and normospermia

(37% vs 4.2% and 1.4% respectively, $p < 0.05$). Moreover, in men with oligospermia, cytogenetic abnormalities were more common in the severe group (7.7%) followed by the moderate (5.25%) and the mild group (3%). The results of the study are by the literature. Karyotyping is suggestive especially in oligospermic /azoospermic men and before proceeding to IVF. The advent of high throughput sequencing technologies and genome-wide association studies will contribute to discovering novel promising genetic factors involved in male infertility.

Keywords
Infertility; oligospermia; azoospermia; chromosomal abnormalities

1. Introduction

Infertility is the failure of a couple to achieve a clinical pregnancy after one year of regular unprotected sexual intercourse, which affects approximately 10-15% of couples globally [1]. About one-third of infertile cases are due to male factors, another third are due to female factors, and the rest are due to both partners or unknown reasons (idiopathic infertility) [1]. Thus, the male partner may be responsible for nearly half of the infertility cases.

Male infertility affects around 7% of the male population and can be caused by genetic, environmental, anatomical, phycological, endocrine and immunological factors [1, 2]. Genetic causes are responsible for nearly 10-15% of male infertility and are associated with impaired spermatogenesis, defective sperm function, or defects in sperm delivery [2]. The most common genetic factors are chromosomal abnormalities, single gene mutations and Y chromosomal microdeletions.

It has been well established that the prevalence of chromosomal abnormalities is strongly correlated with male infertility and is inversely related to sperm concentration, being 5-7% in males with oligospermia and increasing up to 10-15% in patients with azoospermia [3, 4]. Chromosomal abnormalities can be either numerical or structural and involve mainly sex chromosomes, but numerous autosomal anomalies are also detected [3, 4].

This study aimed to investigate the prevalence and distribution of chromosomal abnormalities in the peripheral blood of infertile Greek men subjected to *In Vitro* Fertilization (IVF).

2. Materials and Methods

Four hundred eighty-eight infertile men undergoing IVF at the "GENESIS Athens" clinic were included in the study between June 2016 and June 2022 (mean age = 41.7 years; range: 25-69). They all underwent a complete clinical examination and semen analysis was conducted in conformity with World Health Organization (WHO) recommendations [5]. Subjects were categorized into groups according to their sperm concentrations: 27 had azoospermia (absence of spermatozoa in the ejaculate), 168 had oligospermia (98 mild: 5-15 × 10^6 spermatozoa/ml, 57 moderate: 1-5 × 10^6 spermatozoa/ml and 13 severe oligospermia: <1 × 10^6 spermatozoa/ml) and 293 had normospermia (>15 × 10^6 spermatozoa/ml). Men with infertility due to obstructive azoospermia or other reasons

(environmental or radiation exposure, drugs etc.) were excluded. In all cases, the karyotypes of their female partners were normal (46, XX).

The control group included 38 men (mean age = 32.3 years; range: 19-63) referred for cytogenetic analysis, who were either male donors with normal sperm parameters, or men with proven paternity without assisted reproductive techniques.

The research was performed by the Helsinki Declaration (1975). All participants consented to their anonymous and voluntary participation in the study. The study was approved by the Bioethics and Ethics Committee of the Scientific Board of the GENESIS ATHENS Clinic SA (192/13-6-2016).

2.1 Cytogenetic Analysis

Chromosome preparations from PHA (phytohaemagglutinin)-stimulated peripheral blood lymphocytes (>550 bands per haploid set-bphs) were analyzed using GTG-banding in line with standard cytogenetic protocols. Twenty metaphases were fully analyzed for each case, while on the occasion of suspected mosaicism, 100 metaphases were analyzed. Chromosomal polymorphisms including large satellites, increased heterochromatic regions and small pericentric inversions were not noted. Karyotypes were described according to the International System for Human Cytogenomic Nomenclature 2016 and 2020 [6, 7].

2.2 Statistical Analysis

The SPSS (Statistical Package for the Social Sciences) version 20 software was applied for the Statistical data analysis. Pearson Chi-square and Fisher's exact tests were used to evaluate the relationship between chromosomal abnormalities and sperm concentration. A p-value <0.05 was considered significant.

3. Results

467 out of 488 men tested (95.7%) had a normal karyotype, while in 21 cases (4.3%) chromosomal abnormalities were identified. No chromosomal abnormality was detected in the control group. Sex chromosome abnormalities were detected in 13 cases (2.7%) and autosomal abnormalities in 8 cases (1.6%) (Table 1).

Table 1 Chromosome abnormalities detected in 488 men undergoing IVF.

Chromosomal abnormality	***No (%)***
Sex chromosome abnormalities	**13 (2.7)**
47,XXY (Klinefelter syndrome)	7 (1.5)
47,XXY/46,XY (Mosaic KS)	3 (0.6)
46,XX (sex reversal)	2 (0.4)
46,X,t(Y;17)(q11.23;q23.1)	1 (0.2)
Autosomal abnormalities	**8 (1.6)**

Reciprocal translocation	**5 (1)**
46,XY,t(8;12)(q21.3;p12.3)	1 (0.2)
46,XY,t(2;12)(q31.2;p12.1)	1 (0.2)
46,XY,t(5;7)(q23;q36)	1 (0.2)
46,XY,t(2;8)(q12;p23)	1 (0.2)
46,XY,t(2;14)(p21;q32.3)	1 (0.2)
Robertsonian translocation	**1 (0.2)**
45,XY,der(13;14)(q10;q10)	1
Inversion	**1 (0.2)**
46,XY,inv(20)(p11.21q13.13)	1
Inversion and translocation	**1 (0.2)**
46,XY,inv(6)(p12q24),t(8;9)(q24.1;q32)[7]/46,XY[193]	1
Total	**21 (4.3)**

The correlation of chromosomal abnormalities with sperm concentrations is presented in Table 2. 37% of chromosomal aberrations were detected in men with azoospermia, significantly higher than in those with oligospermia (4.2%) and normospermia (1.4%) ($\chi^2 = 41.92$ and $\chi^2 = 75.19$, df = 1, $p < 0.05$ respectively). Among the different groups of oligospermic men, cytogenetic abnormalities were more common in the severe group (7.7%), then in the moderate group (5.25%) and lastly in the mild group (3%).

Table 2 Correlation of chromosome abnormalities with sperm concentrations men.

Chromosome Abnormality	**Azoospermia (n = 27)**	**Oligospermia (n = 168)**			**Normospermia (n = 293)**
		Mild (n = 98)	**Moderate (n = 57)**	**Severe (n = 13)**	
Sex chromosomes	10	1	1	-	1
Klinefelter Syndrome	7/27 (25.9)	-	-	-	-
Mosaic KS	-	1/98 (1%)	1/57 (1.75%)	-	1/293 (0.35%)
Sex reversal	2/27 (7.4%)	-	-	-	-
Y-autosome translocation	1/27 (3.7%)				
Autosomes	-	2	2	1	3
Reciprocal translocations	-	2/98 (2%)	1/57 (1.75%)	1/13 (7.7%)	1/293 (0.35%)

Robertsonian translocations	-	-	1/57 (1.75%)	-	-
Inversion	-	-	-	-	1/293 (0.35%)
Inversion and translocation	-	-	-	-	1/293 (0.35%)
Total	10/27 (37%)	7/168 (4.2%) 3/98 (3%)	3/57 (5.25%)	1/13 (7.7%)	4/293 (1.4%)

4. Discussion

In the literature, the frequency of chromosomal abnormalities detected in infertile men ranges between 2.1-28.4%, probably due to various sample selection criteria and differences in the methodological approaches, such as the number of cells analyzed and the inclusion or not of chromosomal "variants" [3, 8-12]. The present study confirms the increased frequency of chromosomal abnormalities in infertile men compared to the control group (4.3% vs. 0%). Chromosomal anomalies impair spermatogenesis due to the altered meiotic behavior of the affected chromosomes which depends on the morphology and length of the chromosome fragments involved, the presence or not of aggregated heterochromatin, the frequency of exchanges in the pairing, the interstitial regions and the localization of breakpoints [13].

Chromosomal abnormalities were more common in azoospermic males (37%), than in severe, moderate and mild oligospermic ones with frequencies being 7.7%, 5.25% and 3% respectively. Anomalies were less common in normospermic men (1.4%) confirming the increasing frequency with declining sperm count [3, 4, 8, 14, 15]. Sex chromosome abnormalities were more frequent (2.7%) and had a higher incidence in azoospermic men (37% vs. 2.75% in oligospermic men). In comparison, autosomal chromosome abnormalities had a lower frequency (1.8%) and were more frequently observed in men with oligospermia (4.2% vs. 0% in azoospermic men). These findings are by the literature, where sex chromosome aberrations are detected in 0.4-12.3% of infertile men and are predominant in azoospermic men, while autosomal chromosome abnormalities have been found in a range of 1.1-7.2% of infertile men and are mostly detected in patients with oligospermia [3, 8-12, 15].

It is well known that Klinefelter syndrome (KS) is the most frequent sex chromosome aneuploidy in males, occurring in 0.1-0.2% of newborn males and its prevalence increases up to 2-5% in men with severe oligospermia and up to 15% in azoospermic men [3, 14-16]. In the present study, KS was the most common sex chromosome abnormality (2.1%). 7/10 cases were in a non-mosaic form and were all detected in azoospermic men. 3/10 cases were in a mosaic form and were identified in 2 oligospermic and one normospermic man.

Another sex chromosomal alteration detected was a 46,XX karyotype detected in 2 azoospermic (0.4%) men, with normal phenotypes, due to the translocation of the SRY (Sex Determining Region) gene, onto one X chromosome [17]. 46,XX male sex reversal (de la Chapelle syndrome) is a rare genetic syndrome occurring in about 1/20.000-25.000 newborn males and accounts for 2% of male infertility cases [17-19]. In addition, a Y; autosome translocation was identified in one azoospermic man (0.2%). Y; autosome translocations are rare (~1/2000 newborn males) and they may cause

infertility when the breakpoint is within the Yq11 critical segment where the AZF (Azoospermia Factor) locus is located or near the pseudoautosomal segment (Yp) [3, 8].

Carriers of balanced autosomal rearrangements are phenotypically normal. However, they have an increased risk of producing unbalanced gametes due to spermatogenesis impairment, thus resulting in infertility, miscarriage or the birth of a malformed child. In the present study, all autosomal rearrangements were balanced and detected only in men with oligospermia and normospermia. Reciprocal translocations were more frequent (1%), followed by a Robertsonian translocation 13;14 (0.2%), an inversion (0.2%) and a complex rearrangement having both inversion and translocation (0.2%). No data were available regarding previous miscarriages or not of their partners. According to the literature, reciprocal and Robertsonian translocations have been reported to be 5-7x and 8-9x higher respectively in infertile men compared to the general population and pericentric inversions 8x higher [3, 4, 8, 13].

Presently, a genetic diagnosis is achieved in only 4% of all infertile males [20]. In the current study, only seven men have also tested for Y chromosomal (AZF) microdeletions with one being positive. Thus, a genetic diagnosis was possible in 22/488 (4.5%) men. In about 40% of male infertile patients, the etiology is still unidentified after performing available genetic tests and excluding all known possible acquired diseases (idiopathic male infertility) [2].

The advent of high throughput technologies, like Next-Generation Sequencing, and genome-wide association studies have led to the discovery of several novel promising candidate genes responsible for male infertility, which after validation, could serve as biomarkers for the development of a gene panel-based diagnostic testing in the future [20]. Recently, the first study performed in teratozoospermic Greek patients using Whole Genome Sequencing confirmed the role of already known genes involved in male infertility and enlisted several candidate genes and variants that are associated with teratozoospermia [21]. Epigenetic changes such as DNA methylation, histone modification and non-coding RNA have also been shown to significantly contribute to male infertility. It is suggested that more information could be assessed simultaneously through a new 'omics' approach at different levels [2, 22-24].

5. Conclusions

Diagnosing the genetic defect of male infertility provides clinically important information for appropriate genetic counseling. The present study indicates that karyotyping is far from being outdated for the genetic diagnosis of male infertility and highlights its importance especially in men with oligospermia/azoospermia and before proceeding to IVF. It is believed that new technologies will elucidate the unexplained genetic etiology and provide new biomarkers for diagnosing male infertility.

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Author Contributions

EK conceived, designed and coordinated the study; EK, HT and SZ contributed to cytogenetic analyses and interpretation of the results; EK, CK, SS and AP performed data collection and analysis; NN performed semen analyses; LL performed molecular analyses; EK and CK contributed to manuscript writing; EK, AM contributed to manuscript review and editing. All authors read and approved the final manuscript.

Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

Please note that all data of the study are held in our premises in accordance of the provisions of the applicable legislation and may be accessible by you on a codified basis upon request.

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