ORIGINAL ARTICLE

Prevalence and Spectrum of Chromosomal Abnormalities in 1366 Infertile Tunisian Men: A retrospective observational study

Prévalence et spectre des anomalies chromosomiques chez 1366 hommes tunisiens infertiles : Etude observationnelle rétrospective

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Abstract

Introduction: Chromosomal abnormalities are a well-documented cause of male infertility, particularly in patients with severe sperm defects such as azoospermia.
Aim: To assess the prevalence and types of chromosomal abnormalities in infertile men referred for cytogenetic analysis over a 19-year period, and to explore genotype-phenotype correlation.
Methods: We retrospectively analyzed 1366 karyotypes performed between 2006 and 2024 in patients with abnormal semen parameters, referred at the Genetics Department of Charles Nicolle Hospital in Tunis for genetic investigations. Data were collected from medical records and laboratory reports. Standard karyotyping was performed in all cases, supplemented by fluorescence in situ hybridization (FISH) and Y chromosome microdeletion analysis when indicated.
Results: A total of 117 chromosomal abnormalities were identified among the 1366 infertile men, corresponding to a global prevalence of 8.5%. The most frequent clinical presentation was azoospermia, accounting for 60.3% of patients with chromosomal abnormalities. Numerical chromosomal abnormalities accounted for 80.3% (n = 94) of all detected cases. The most prevalent was Klinefelter syndrome, found in 85 patients, representing 72.6% of all anomalies. Among patients with Klinefelter syndrome, 96.4% had non-obstructive azoospermia. Structural abnormalities were observed in 23 patients (19.6%), comprising balanced translocations, inversions, Robertsonian translocations, and unbalanced chromosomal anomalies such as Yq deletions and supernumerary marker chromosomes.
Conclusion: Cytogenetic abnormalities were detected in nearly 8.5% of infertile men. These findings support the utility of systematic karyotype.
Key words: Azoospermia, Chromosomal abnormalities, Cytogenetics, Klinefelter syndrome, Male infertility, Oligospermia

Résumé

Introduction: Les anomalies chromosomiques sont une cause bien documentée d'infertilité masculine, en particulier chez les patients présentant des malformations spermatozoïdes sévères telles que l'azoospermie. L'objectif de cette étude est d'évaluer la prévalence et les types d'anomalies chromosomiques chez les hommes infertiles soumis à une analyse cytogénétique sur une période de 19 ans, et d'explorer la corrélation génotype-phénotype.
Méthodes : Nous avons analysé rétrospectivement 1366 caryotypes réalisés entre 2006 et 2024 chez des patients présentant des paramètres de sperme anormaux, orientés au département de génétique de l'hôpital Charles Nicolle à Tunis pour des recherches génétiques. Les données ont été recueillies à partir de dossiers médicaux et de rapports de laboratoire. Le caryotypage standard a été réalisé dans tous les cas, complété par une hybridation fluorescente in situ (FISH) et une analyse de microdélétion du chromosome Y lorsque cela est indiqué.
Résultats : Un total de 117 anomalies chromosomiques ont été identifiées parmi les 1366 hommes infertiles, correspondant à une prévalence mondiale de 8,5 %. La présentation clinique la plus fréquente était l'azoospermie, représentant 60,3 % des patients présentant des anomalies chromosomiques. Les anomalies chromosomiques numériques représentaient 80,3 % (n = 94) de tous les cas détectés. Le plus fréquent était le syndrome de Klinefelter, observé chez 85 patients, représentant 72,6 % de toutes les anomalies. Parmi les patients atteints du syndrome de Klinefelter, 96,4 % présentaient une azoospermie non obstructive. Des anomalies structurelles ont été observées chez 23 patients (19,6 %), comprenant des translocations équilibrées, des inversions, des translocations robertsoniennes et des anomalies chromosomiques déséquilibrées telles que des délétions Yq et des chromosomes marqueurs surnuméraires.
Conclusion : Des anomalies cytogénétiques ont été détectées chez près de 8,5 % des hommes infertiles. Ces résultats confirment l'utilité du caryotype systématique.
Mots clés: Azoospermie, aberrations chromosomiques, cytogénétique, syndrome de Klinefelter, infertilité masculine, oligospermie.

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 DOI: 10.62438/tunismed.v104i01.6262

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Introduction

Infertility is defined by the World Health Organization (WHO) as the inability of a couple to conceive or carry a pregnancy to term after one year or more of regular unprotected sexual intercourse. It is a major public health issue, affecting approximately 10 to 15% of couples worldwide. Around half of cases are due to female factors, while 20-30% are caused by male factors, with the remaining cases involving both partners (1). Male infertility is classified as either primary, in the absence of any prior conception, or secondary, when infertility occurs after a previous conception. Etiologically, it is divided into secretory forms, due to impaired spermatogenesis, and excretory forms, resulting from obstruction of the seminal pathways (2). This study focuses exclusively on cases of secretory infertility.

Male infertility encompasses a wide range of phenotypes including abnormalities in sperm count, motility, or morphology. These alterations can be related to environmental, endocrine, or genetic factors. Around 15% of male infertility are attributable to genetic causes. About 8% are due to chromosomal anomalies, frequently associated with significant spermatogenic defects (3). These abnormalities may involve chromosome number (e.g., aneuploidies) or structure (e.g., translocations, deletions).

With the development of assisted reproductive technologies (ART), particularly intracytoplasmic sperm injection (ICSI), identifying the genetic basis of male infertility has become crucial. Performing a karyotype, combined with targeted molecular analyses, helps clarify the etiology, guide management strategies, predict ART outcomes, and provide appropriate genetic counseling. In Tunisia, several studies have investigated the contribution of chromosomal abnormalities to male infertility, across cohorts of up to 1,000 patients (4-8).

Here, we present the largest Tunisian series to date, based on a cytogenetic study conducted at the Department of Genetics of Charles Nicolle Hospital in Tunis, a national reference center for medical genetics. This study expands the existing dataset of Tunisian patients and contributes to a deeper understanding of the genetic landscape of male infertility in the population. Furthermore, we aim to establish genotype-phenotype correlations for the main chromosomal abnormalities identified, in order to optimize clinical management and genetic counseling.

Methods

This retrospective, single-center observational study was conducted over a 19-year period, from January 2006 to December 2024, at the Genetics Department of Charles Nicolle Hospital in Tunis. It included infertile males with abnormal semen parameters of presumed non-obstructive origin who were referred for genetic investigation. Patients lacking semen parameters were excluded from the study. Epidemiological, clinical, and biological data were collected from medical records and laboratory reports. All genetic investigations were performed after obtaining informed consent from the patients. Results were communicated during individual genetic counseling consultations.

A standard peripheral blood karyotype was systematically performed as a first-line test for all included patients. After cell culture and R-banding, metaphase chromosomes were examined under a light microscope, captured using an integrated camera system, and subsequently arranged for analysis. Fifteen mitoses were analyzed for each patient. In cases of mosaicism, up to fifty metaphases were examined and a threshold of 5% was used to consider mosaicism as significant and potentially responsible for the phenotype. Structural benign polymorphic variants, such as heterochromatic variants (e.g., 1qh+, 9qh+, Yqh+), satellite variations of acrocentric chromosomes, certain common inversion (e.g., inv(5)(p13q13), inv(9)(p11q12), inv(2)(p11.2q13)) and commun fragile sites, were not classified as pathogenic and were interpreted as normal variants. When necessary, additional analysis using fluorescence in situ hybridization (FISH) was performed to better characterize certain chromosomal abnormalities detected by karyotype.

A multiplex PCR screening for Y-chromosome microdeletions involving the azoospermia factor (AZF) loci (AZFa, AZFb, and AZFc) was performed in patients with Yq deletions to determine whether the AZF regions were absent. PCR amplification of SRY gene was performed in cases with a 46,XX karyotype. An array comparative genomic hybridization (array CGH) study was carried out in one 46,XX/SRY-negative patient. Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) software, version 21. Qualitative variables were expressed as counts and percentages, while quantitative variables were presented as mean ± standard deviation for normally distributed parameters, and as median [min-max] for non-normally distributed parameters.

Results

Between 2006 and 2024, a total of 25,644 karyotypes were performed at our laboratory. Among them, 1366 cases (5.3%) were referred for abnormal semen parameters. Of these, 824 cases (60.3%) involved azoospermia, 506 (37 %) oligo-astheno-teratozoospermia (OATS), 21 (1.5%) cryptozoospermia, 9 (0.6%) oligospermia and 6 cases (0.4%) isolated teratozoospermia. Overall, 117 chromosomal abnormalities were identified (8.5%). Within this group, the median age at the first genetic consultation was 38 years (range: 27-51 years), with the majority of patients (73 out of 117; 62.4%) belonging to the age group 31-40 years. The median duration of infertility was four years, ranging from one to 31 years. Nearly all patients (99.8%) had no history of conception. Only one patient had a child from a first marriage, and another had experienced a spontaneous pregnancy loss. Based on semen analysis (spermogram/spermocytogram) results, patients were classified into two categories of sperm abnormalities: quantitative abnormalities observed in 116 out of 117 patients (99.1%), and qualitative abnormalities found in one patient. Quantitative

abnormalities included azoospermia, the most frequent condition, identified in 100 patients (85.5%), followed by OATS in 14 patients (12%) and cryptozoospermia in two patients (1.7%). Regarding qualitative abnormalities, the single case involved semen degradation associated with asthenozoospermia and necrozoospermia (Table 1).

Table 1. Distribution of sperm abnormalities in patients with chromosomal abnormalities

Type of Sperm Abnormality	Number of Patients	Percentage (%) (N°/117*)	Percentage (%) (N°/1366*)
Quantitative Abnormalities	**116**	**99.1**	**8.5**
Azoospermia	100	85.5	7.3
OATS	14	12	1
Cryptozoospermia	2	1.7	0.1
Qualitative Abnormality	**1**	**0.9**	**0.07**
Degraded sperm + Asthenospermia + Necrozoospermia	1	0.9	0.07

*117: Total of chromosomal abnormalities, 1366: Total of infertile men, OATS: Oligo-astheno-teratozoospermia

A detailed overview of the chromosomal abnormalities identified in our cohort is presented in Table 2. Numerical abnormalities were observed in 94 out of 1366 infertile patients (7%) and accounted for 80.3% of the chromosomal abnormalities detected. However, structural abnormalities were identified in 23 patients (1.7%), representing 19.6 % of the chromosomal abnormalities. Klinefelter syndrome (47,XXY) and its variants represented the most frequent abnormality, identified in 85 of the 117 patients with chromosomal abnormalities (72.6%) and in 10.3% of the 824 azoospermic patients.

Among the 85 patients with Klinefelter syndrome, 77 (90.6%) had the homogeneous form and 8 (9.4%) presented mosaicism with variable proportions of 47,XXY cells. Overall, azoospermia was observed in 96.5% (82/85) of cases. In the homogeneous subgroup (n=77), 75 patients (97.4%) had azoospermia, while two exhibited severe OATS.Among patients with mosaicism, seven had azoospermia and one presented with OATS.

Of the 117 patients with chromosomal abnormalities, five (4.3%) carried an extra Y chromosome, consistent with 47,XYY syndrome. One of these patients presented with azoospermia, while the remaining four had OATS. Notably, one OATS patient showed a mosaic karyotype: 45,X[6]/47,XYY[4]/46,XY[60].

Three of the 117 patients (2.6 %) had a karyotype compatible with a disorder of sex development (DSD). Two patients had a female karyotype (46,XX) consistent with 46,XX testicular DSD. Molecular and FISH analyses analysis revealed the presence of the SRY gene on the X chromosome in one patient and an upstream duplication of the SOX9 gene in the other. A third patient had a mosaic karyotype: mos 46,XY[17]/46,XX[13].

Structural chromosomal abnormality was identified in 23 out of 1366 patients (1.7%), including 14balanced structural abnormalities (1%): seven reciprocal translocations (0.5%), five robertsonian translocations (0.4%) and two pericentric inversions (0.1%). Six of the seven reciprocal translocations involved autosomes, and one was an X-autosome translocation.

Among the patients with reciprocal translocations, three presented with azoospermia, two with cryptozoospermia, and two with OATS. Among Robertsonian translocation carriers, three had azoospermia, one had OATS, and one showed semen degradation. Patients with pericentric inversions had both OATS.

Unbalanced structural abnormalities were identified in 9 out of 1366 patients (0.65%). Three involved a terminal deletion of the Y chromosome, three cases showed mosaicism with an isochromosome of the Y short arm and three presented a supernumerary marker chromosome (SMC).

Y chromosome microdeletion analysis (AZF loci) revealed a combined deletion of the AZFb and AZFc regions in the three patients with a terminal Y chromosome deletion and in one patient with mosaicism 45,X/46,X,idic(Yq).

Overall, chromosomal abnormalities were found in 8.5% of infertile men, with Klinefelter syndrome being the most frequent (72.6% of all abnormalities). Structural rearrangements, although less common (19.6% of cases), included both balanced and unbalanced forms, often associated with severe spermatogenic impairment.

Discussion

In our cohort, a genetic etiology explaining the sperm abnormalities was identified in 117 patients (8.5%). This result is consistent with data from the literature. Indeed, previous studies conducted across various populations have reported an incidence of chromosomal abnormalities in infertile men ranging from 4.3% to 19.73% (6,9). The discrepancies between studies may result from several factors, such as geographic origin, sample size, inclusion criteria, and the types of chromosomal abnormalities considered causative. Overall, chromosomal abnormalities detected by karyotyping are observed in 2-8% of infertile men. The prevalence increases to approximately 15% among men with azoospermia, which is 10-20 times higher than in the general population (3). Table 3 summarizes the findings of studies on the prevalence of chromosomal abnormalities in infertile men in Tunisia with a reported range between 8% (5) and 19% (6).

Klinefelter syndrome was the most frequent chromosomal abnormality, accounting for 85 of the 117 anomalies (72.6%) and corresponding to a prevalence of 10.3% among azoospermic patients (85/824). This observation is consistent with the findings of Ferlin et al., who reported rates up to 5% in severe oligozoospermia group and 10% in azoospermia group (3). Among patients with Klinefelter syndrome, 96.5% (82/85) had azoospermia and 90.6% (77/85) a homogeneous karyotype (Table 2). These results are consistent with previous reports, indicating that more than 90% of Klinefelter patients present with azoospermia and have a homogeneous karyotype(6).

Table 2. Types and Frequencies of Chromosomal Abnormalities: Correlation with Sperm Phenotype

Chromosomal Abnormalities	% (N°/ Total Chromosomal Abnormalities)	% (N°/Total Infertile Men)	Sperm Phenotype (N°)
Numerical Abnormalities	80.3 (94/117)	7 (94/1366)	
47,XXY	65.8 (77/117)	5.6 (77/1366)	Azoospermia (75) / OATS (2)
Mosaic 47,XXY	6.8 (8/117)	0.6 (8/1366)	
47,XXY[38]/46,XX[12]	0.8 (1/117)	0.07 (1/1366)	Azoospermia
47,XXY[21]/48,XXXY[1]	0.8 (1/117)	0.07 (1/1366)	Azoospermia
47,XXY[29]/48,XXXY[1]	0.8 (1/117)	0.07 (1/1366)	Azoospermia
47,XXY[29]/49,XXXXY[1]	0.8 (1/117)	0.07 (1/1366)	Azoospermia
47,XXY[2]/47,XYY[1]/46,XY[32]	0.8 (1/117)	0.07 (1/1366)	OATS
47,XXY[48]/48,XXXY[1]/46,XY[1]	0.8 (1/117)	0.07 (1/1366)	Azoospermia
47,XXY[44]/46,XX[3]/49,XXXXY[1]/48,XXXY[1]/46,XY[1]	0.8 (1/117)	0.07 (1/1366)	Azoospermia
47,XXY[34]/46,XY[11]/48,XXYY[2]/49,XXXXY[2]/50,XXXXYY[1]	0.8 (1/117)	0.07 (1/1366)	Azoospermia
47,XYY	4.3 (5/117)	0.4 (5/1366)	Azoospermia (1) / OATS (4)
45,X[6]/47,XYY[4]/46,XY[60]	0.8 (1/117)	0.07 (1/1366)	OATS
Males 46,XX	2.5 (3/117)	0.2 (3/1366)	
46,XX	1.7 (2/117)	0.1 (2/1366)	Azoospermia (2)
46,XY[17]/46,XX[13]	0.8 (1/117)	0.07 (1/1366)	OATS
Structural Abnormalities	19.6 (23/117)	1.7 (23/1366)	
Reciprocal Translocations	6 (7/117)	0.5 (7/1366)	
46,XY,t(X;19)(p11.2;p12)	0.8 (1/117)	0.07 (1/1366)	OATS
46,XY,t(3;7)(q21;p21)	0.8 (1/117)	0.07 (1/1366)	Azoospermia
Chromosomal Abnormalities	% (N°/ Total Chromosomal Abnormalities)	% (N°/Total Infertile Men)	Sperm Phenotype (N°)
46,XY,t(1;14)(p31;q31)	0.8 (1/117)	0.07 (1/1366)	Azoospermia
46,XY,t(12;16)(q21;q21)pat	0.8 (1/117)	0.07 (1/1366)	OATS
46,XY,t(12;13)(q12;p11.2)	0.8 (1/117)	0.07 (1/1366)	Azoospermia
46,XY,t(11;22)(q12;q11.2)	0.8 (1/117)	0.07 (1/1366)	Cryptozoospermia
46,XY,t(10;21)(p11.21;p11.2)	0.8 (1/117)	0.07 (1/1366)	Cryptozoospermia
Robertsonian Translocations	4.3 (5/117)	0.4 (5/1366)	
45,XY,rob(13;14)(q10;q10)	3.4 (4/117)	0.3 (4/1366)	Azoospermia (2) /OATS (1) / Sperm degradation (1)
45,XY,rob(14;15)(q10;q10)	0.8 (1/117)	0.07 (1/1366)	Azoospermia
Pericentric inversions	1.7 (2/117)	0.1 (2/1366)	
46,XY,inv(7)(p21q21)	0.8 (1/117)	0.07 (1/1366)	OATS
46,XY,inv(12)(p12q24.1)pat	0.8 (1/117)	0.07 (1/1366)	OATS
Supernumerary marker chromosomes	2.6 (3/117)	0.2 (3/1366)	
47,XY,+mar.ish der(14/22)(D14Z1/D22Z1+)	1.7 (2/117)	0.1 (2/1366)	Azoospermia (2)
47,XY,+mar	0.8 (1/117)	0.07 (1/1366)	Azoospermia
Chromosome Y rearrangement	5.1 (6/117)	0.4 (6/1366)	
46,X,del(Y)(q11.222)	2.6 (3/117)	0.2 (3/1366)	Azoospermia (3)
45,X[34]/46,X,idic(Y)(q11.2)[16]	0.8 (1/117)	0.07 (1/1366)	Azoospermia
46,X,idic(Y)(q11.2)[47]/45,X[3]	0.8 (1/117)	0.07 (1/1366)	Azoospermia
46,X,idic(Y)(q11.222)[26]/45,X[8]	0.8 (1/117)	0.07 (1/1366)	Azoospermia

OATS: Oligo-astheno-teratozoospermia

Table 3. Comparison of chromosomal abnormality frequencies in Tunisian studies

Tunisian studies	Number of Cases	Number of Karyotype Abnormalities (N°/ total)	Frequency (%)
Elghezal et al. , 2006 [8]	1000	135/1000	13.5
Ghorbel et al., 2012 [6]	76	15/76	19.7
Amouri et al., 2014 [4]	476	52/476	10.9
Chabchoub et al., 2019 [5]	84	7/84	8.3
Our study	1366	119/1366	8.7

Interestingly, two patients with a homogenous 47,XXY karyotype presented with severe oligozoospermia. This observation aligns with the findings of Foresta et al., who reported that residual spermatogenesis may occur in 47,XXY individuals, with spermatogonia capable of completing the spermatogenic process and generating mature spermatozoa (10).

A 47,XYY syndrome was identified in six patients (0.4%), five homogeneous and one mosaic form (45,X[6]/47,XYY[4]/46,XY[60]) (Table 2), a rate comparable to that reported by Abur et al. in 2019 (11). Unlike Klinefelter syndrome, sperm parameters in men with 47,XYY syndrome are highly variable and may be normal (11).

Two patients in our cohort were diagnosed with 46,XX DSD (2/1366, 0.14%). The 46,XX male syndrome is characterized by a male phenotype despite a 46,XX chromosomal constitution. Histologically, it is associated with the absence of spermatogenesis, primarily due to the lack of the AZF loci located on Yq11.221-11.223 (12). Both our patients presented azoospermia, in in line with previous reports. The male phenotype in these patients is attributed, in more than 80% of cases, to the presence of the SRY gene, typically translocated to the X chromosome or an autosome. In our cohort, molecular and FISH analyses confirmed the presence of the SRY gene translocated to the X chromosome in one patient. However, the second patient was SRY-negative, with a ~500 kb duplication identified by array CGH upstream of the SOX9 gene (13). Additionally, one patient with a mosaic karyotype 46,XY[17]/46,XX[13] presented with OATS (Table 2), which may be explained by the presence of a 46,XY cell line. Notably, patients with 46,XX/46,XY mosaicism may present with external genitalia ranging from typical male to ambiguous to typical female (14).

Structural chromosomal abnormalities, such as deletions, translocations, and inversions, are found in approximately 5% of infertile men (1). In our study, they were observed in 1.7% (23/1366) of patients. This lower prevalence compared to the literature is most likely explained by the exclusion of certain anomalies currently considered common polymorphic variants.

Y chromosome structural abnormalities are the most frequently observed chromosomal anomalies in infertile men after Klinefelter syndrome. These alterations include isodicentric Y chromosomes, ring Y chromosomes, or deletions of various sizes, each associated with a range of phenotypic consequences (15). Three azoospermic patients (0.2%) had a Yq deletion detected by Karyotype, consistent with reported rates (0.23-0.53%) (15,16). All showed combined AZFb/AZFc deletions.

In addition, three azoospermic patients (0.2%) carried a mosaic isochromosome of Yp (Table 2), similar to the 0.3% reported by Yoshida et al. (0.3%) (17).

Among the structural abnormalities identified in our cohort, the proportion of reciprocal translocations and robertsonian was 0.5%, and 0.3% respectively. These rates are slightly lower than those typically reported in the literature. Hajlaoui et al. (7) reported a 0.9% prevalence of Robertsonian translocations among infertile men, higher in oligospermia than in azoospermia. The most frequent robertsonian translocations are rob(13;14) (73-85%), followed by rob(14;21) (10%). In our cohort, four out of five cases were rob(13;14), while one was rob(14;15). Most patients were azoospermic (3/5) (Table 2), consistent with the predominance of azoospermic patients referred for karyotype analysis.

Seven patients carried reciprocal translocations: six autosome-autosome translocations and one X-autosome translocation. Three patients presented with azoospermia, two with OATS and two with cryptozoospermia (Table 2). According to Siffroi et al., structural anomalies interfere with normal chromosome behaviour at meiosis and lead to germ cell breakdown (18). At the cellular level, apoptosis is characterized by the activation of an enzymatic cascade resulting in double-stranded DNA fragmentation, which can be assessed in human sperm. Du et al. demonstrated a significant increase in sperm DNA fragmentation in patients with rob(13;14) compared to men with a normal karyotype, consistent with the sperm degradation observed in our patient with rob(13;14) (19).

Additionally, an alternative mechanism that could explain the sperm abnormalities observed in cases of reciprocal translocations is the disruption or altered expression of genes essential for spermatogenesis located at or near the chromosomal breakpoints (20).

In our cohort, a reciprocal translocation t(1;14)(p31;q31) was identified in a patient with azoospermia. The breakpoints may have disrupted key genes, including MSH4 at 1p31, essential for homologous chromosome pairing and chiasma formation (Spermatogenic failure 2 , OMIM:108420), and SPATA7 at 14q31.3, implicated in germ cell development (21,22). Another patient with cryptozoospermia carried a t(11;22)(q12;q11.2) translocation. The 22q11.2 region harbors the TSSK2 (Testis-Specific Serine/Threonine Kinase 2) gene, encoding a testis specific kinase crucial for sperm maturation. A recent study reported that translocations involving the 22q11.2 breakpoint were associated with oligozoospermia in infertile men and suggested that the breakpoint rearrangement may disrupt spermatogenesis through the involvement of TSSK2 (23)

Inversions are rare in infertile men. In our cohort, two pericentric inversions were identified (chromosome 7 and 12), representing 0.14% of all karyotypes and 1.7% of chromosomal abnormalities. These results are in line with those reported by Guo et al.(2.1%)(24). Both our patients presented with OATS.

Among structural chromosomal abnormalities, two patients with OATS carried balanced rearrangements inherited from their fathers: a pericentric inversion of chromosome 12 (46,XY,inv(12)(p12q24.1)) and a reciprocal translocation (46,XY,t(12;16)(q21;q21)). The paternal origin of these rearrangements may suggest a benign familial variant. However, the variable expression observed between the probands and their fathers may be explained by the presence of modifier factors or meiotic microrearrangements.

The structural anomalies identified in our cohort deserve further investigation using high-resolution techniques such as optical genome mapping, whole-genome sequencing, or long-read sequencing to better define breakpoints and detect potential gene disruptions or positional effects that could account for the phenotype.

In our cohort, three azoospermic patients carried a supernumerary marker chromosome: two der(14/22) and one uncharacterized marker. SMCs occur in ~0.35% of infertile men, mainly from acrocentric chromosomes, most often 14 or 15 (42%) (25). The mechanism proposed is gametogenesis failure most probably induced by a mechanical chromosome difficulty during meiosis (26).

Overall, a chromosomal cause was identified in 8.5% of patients in our cohort. Diagnosis was established primarily by conventional karyotyping, supplemented in some cases with FISH and targeted molecular analyses. Emerging genome-wide technologies, both cytogenetic (e.g., array CGH) and molecular (e.g., Next Generation sequencing, NGS), are increasingly contributing to the identification of genetic causes of infertility through the discovery of novel copy number variants (CNVs) and single nucleotide variants (SNVs). Recent exome-based strategies combining SNV and CNV detection have nearly doubled diagnostic yield (from 10.2% to 19.8%) and continue to expand the list of infertility associated genes (from 78 in 2019 to 120 in 2020) (27).

Our study presents some limitations that should be considered. First, it was conducted in a single center, which may restrict the generalizability of the findings. In this regard, a meta-analysis of different Tunisian single-center studies would provide stronger evidence. Second, its retrospective design limited the availability of detailed semen analysis data in several patient records. Although the sample size is relatively large, it remains insufficient to adequately assess certain rare chromosomal abnormalities. Moreover, our cohort mainly included men with azoospermia, who are more promptly referred for genetic testing due to the strong association with chromosomal abnormalities, particularly Klinefelter syndrome, whereas oligospermic men are usually referred later, often after initial management or failed assisted reproduction attempts. Regarding diagnostic methods, conventional karyotyping and targeted techniques such as FISH or Y-chromosome-specific molecular analyses were used. However, these approaches cannot detect microrearrangements, cryptic translocations, or sequence variants, which require high-resolution technologies such as array CGH or NGS. Another limitation is the absence of reproductive follow-up data, which would have enabled correlation of genetic anomalies with assisted reproduction outcomes and provided clinically relevant insights into prognosis, treatment planning, and the likelihood of success with specific ART strategies. Finally, comparisons with published data should be interpreted with caution, due to methodological differences.

Conclusion

Our findings are consistent with data from the literature, confirming the importance of cytogenetic analyses in the investigation of male infertility. The absence of chromosomal or molecular anomalies in the rest of the cohort does not rule out a genetic etiology. The use of genome wide technologies (CGH array and NGS) in the genetic investigation of semen abnormalities could enhance diagnostic yield in these patients, enabling more precise management and genetic counseling. The application of genome optical mapping may provide further insights into the nature of apparently balanced chromosomal rearrangements (such as certain translocations or inversions) by detecting potential disruptions affecting genes involved in infertility. Future studies should systematically collect ART outcome data to establish correlations with genetic profiles, thereby improving the assessment of reproductive prognosis and transmission risk. These perspectives underscore the importance of combining conventional cytogenetics with genome-wide technologies to deepen our understanding of the genetic basis of male infertility and refine genetic counseling in the era of precision medicine.

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