Medico Research Chronicles
ISSN NO. 2394-3971
DOI No. 10.26838/MEDRECH.2025.12.1.770
Contents available at www.medrech.com

Karyotype Analysis among Selected Bangladeshi Azoospermic Male Infertile Patients

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ARTICLE INFO **ABSTRACT** **ORIGINAL RESEARCH ARTICLE**

Article History
Received: January 2025
Accepted: February 2025
Key Words:
Azoospermia, Male Infertility, Karyotyping, Klinefelter Syndrome, Chromosomal Abnormalities, Bangladesh.

Background: Male infertility is a global health concern, with genetic factors playing a crucial role in its etiology. Azoospermia, the absence of sperm in ejaculate, is often associated with chromosomal abnormalities. Karyotyping is a key diagnostic tool for identifying chromosomal alterations linked to male infertility. This study aimed to evaluate the karyotypic profiles of Bangladeshi azoospermic male infertile patients, highlighting the prevalence and clinical implications of chromosomal abnormalities.

Methods: This cross-sectional descriptive study was conducted at the Genomic Research Laboratory, Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from March 2022 to February 2023. Sixty-four azoospermic male patients aged 21–60 years were recruited through purposive sampling. Karyotyping was performed using the Giemsa banding (G-banding) technique following ISCN (2009) guidelines. Hormonal profiling and ultrasonographic assessments were conducted to evaluate testicular and endocrine abnormalities. Data analysis was performed using IBM SPSS Statistics (version 25).

Results: Among the 64 patients, 6.25% had Klinefelter syndrome (47,XXY), while 93.75% exhibited a normal karyotype (46,XY). Hormonal analysis showed that 6.3% of patients had abnormal testosterone, FSH, and LH levels, while 3.1% had abnormal prolactin and TSH levels. Ultrasonography revealed testicular abnormalities in 12.5% of patients, including bilateral small testes (6.25%) and epididymal cysts (3.1%). Antichlamydia antibodies were detected in 15.63% of patients, suggesting past or ongoing infections.

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Conclusion: This study underscores the importance of karyotyping in azoospermic males, detecting Klinefelter syndrome in 6.25% of cases, aligning with global prevalence rates. Given that 93.75% had a normal karyotype, further genetic screening, including Y-chromosome microdeletion analysis and whole-genome sequencing, is recommended. Integrating karyotyping, molecular diagnostics, and genetic counseling into infertility management can improve clinical decision-making and reproductive outcomes.

2025, www.medrech.com

INTRODUCTION

Infertility is the inability of a couple to conceive after one year of regular, unprotected intercourse [1]. Globally, approximately 15% of couples, or 60–80 million, experience infertility [2]. Males contribute to 35–40% of cases, females to another 35–40%, while 20–30% result from combined or unknown factors [3]. In Bangladesh, 29% of men attending infertility clinics are infertile [4]. Among the general male population, infertility affects around 7%, with genetic disorders responsible for 15% of cases, including chromosomal anomalies in 4.15% [5,6].

Azoospermia, the complete absence of sperm in ejaculate, is a severe form of male infertility often linked to chromosomal abnormalities such as duplications, deletions, translocations, inversions, and insertions [7]. Genetic testing, particularly karyotyping, is essential for detecting chromosomal numerical and structural alterations [8]. However, karyotyping has limited resolution and can only detect abnormalities larger than 5 megabases, such as aneuploidies and translocations [9]. Molecular techniques like polymerase chain reaction (PCR) and gene sequencing are needed to identify smaller mutations and microdeletions [10].

Karyotyping is a key diagnostic tool for detecting chromosomal abnormalities in azoospermic men, guiding fertility treatments such as testicular sperm extraction and intracytoplasmic sperm injection (ICSI) [11]. However, ICSI-conceived offspring may inherit infertility, necessitating genetic counseling [12]. Emerging genetic technologies, including clustered regularly interspaced short palindromic repeats (CRISPR), offer future possibilities for correcting chromosomal abnormalities and improving fertility outcomes [13,14].

This study aims to assess the karyotypic profiles of selected Bangladeshi azoospermic male infertile patients, highlighting the prevalence of chromosomal abnormalities and their implications for reproductive health and genetic counseling.

METHODOLOGY

Study Design: This cross-sectional descriptive study investigated karyotyping among selected Bangladeshi azoospermic male infertile patients. The primary objective was to detect chromosomal abnormalities and assess genetic variations associated with spermatogenic failure, with a specific focus on karyotypic analysis.

Study Setting and Duration: The study was conducted at the Genomic Research Laboratory, Department of Anatomy, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Data collection and laboratory analyses took place between March 1, 2022, and February 28, 2023.

Study Population and Sampling: A total of 64 azoospermic male patients were recruited from the Outpatient Department of Reproductive Endocrinology and Infertility, BSMMU, using purposive sampling. The inclusion criteria required participants to be Bangladeshi residents aged 21–60 years with a

confirmed diagnosis of azoospermia and a history of primary or secondary infertility. Patients with co-morbid conditions such as thyroid dysfunction, diabetes, or hypertension, as well as those with a history of infections, or use of medications known to affect fertility, were excluded from the study. Selection was based on thorough clinical evaluation and semen analysis to ensure the reliability of the findings.

Ethical Considerations: Ethical approval was obtained from the Institutional Review Board (IRB) of BSMMU. Written informed consent was secured from each participant, ensuring confidentiality and anonymity through unique patient ID numbers. The study adhered to the ethical principles outlined in the Declaration of Helsinki (7th revision, 2013).

Laboratory Procedures

Karyotyping Analysis: For karyotyping, 2 mL of venous blood was collected in heparinized tubes and cultured in a medium supplemented with phytohemagglutinin (PHA) to stimulate lymphocyte proliferation. Cultures were incubated at 37°C for 72 hours, with metaphase arrest induced using colchicine solution at the 70th hour. Hypotonic treatment facilitated chromosomal spreading, followed by fixation using Carnoy's fixative (methanol-acetic acid solution). Cells were spread onto glass slides and stained using the Giemsa banding (G-banding) technique. A total of 20 metaphase spreads per patient were analyzed, with 10 images processed using spectral imaging software. Chromosomal analysis followed the International System for Human Cytogenomic Nomenclature (ISCN, 2009) guidelines.

Data Analysis: Statistical analyses were conducted using IBM SPSS Statistics (version 25, 2017). Descriptive statistics, including mean, standard deviation, and frequency distributions, were used to summarize demographic and laboratory findings. Chromosomal abnormalities identified in the study were compared with published genetic databases for clinical correlation.

Quality Control and Assurance: All laboratory procedures were conducted in accordance with standard operating protocols (SOPs) to ensure accuracy and reliability. To prevent contamination, procedures were performed in a Class II biosafety cabinet under sterile conditions. Pipettes and reagents were validated before use to maintain precision and consistency in results. Additionally, any borderline or ambiguous karyotyping findings were reanalyzed to verify accuracy and eliminate potential errors.

RESULTS

Table 1: Sociodemographic Characteristics of Male Infertile Patients

Characteristic	Number of Patients (n = 64)	Percentage (%)
Age at Diagnosis		
21-30 years	20	31.3
31-40 years	36	56.3
41-50 years	8	12.5
BMI		
Normal (18.5–25)	34	53.1
Overweight (25-30)	26	40.6
Obese (>30)	4	6.25
Education		
Secondary School	38	59.38
Higher Education	26	40.63

Occupation		
Service Holder	26	40.63
Businessman	24	37.5
Worker (e.g., factory)	14	21.88
Exposure History		
Positive	8	12.5
Negative	56	87.5
Family History of Infertility		
Positive	4	6.25
Negative	60	93.75

Table 1 presents the sociodemographic characteristics of 64 male infertile patients. The majority of patients (56.3%) were diagnosed between the ages of 31–40, followed by 31.3% diagnosed between 21–30 years, and 12.5% diagnosed in the 41–50 years range. Regarding BMI, most patients had a normal weight (53.1%), with 40.6% classified as overweight and 6.25% obese. In terms of education, 59.38% had completed secondary school, and 40.63% had higher education. Occupationally, 40.63% were service holders, 37.5% were businessmen, and 21.88% worked in various labor-intensive jobs. Exposure to multiple sexual partners was reported in 12.5% of cases, and 6.25% of patients had a positive family history of infertility, indicating a relatively low genetic influence in this cohort.

Table 2: Hormone Profile of Male Infertile Patients

Hormone Level	Normal (n = 60)	Percentage (%)	Abnormal (n = 4)	Percentage (%)
Serum Testosterone	60	93.8	4	6.3
Serum TSH	62	96.9	2	3.1
Serum FSH	60	93.8	4	6.3
Serum LH	60	93.8	4	6.3
Serum Prolactin	62	96.9	2	3.1

Table 2 illustrates the hormone profiles of the male infertile patients in this study. The majority of patients had normal levels of key hormones: 93.8% for serum testosterone, FSH, and LH, and 96.9% for serum prolactin and TSH. Only a small percentage of patients showed abnormal hormone levels—6.3% for testosterone, FSH, and LH, and 3.1% for prolactin and TSH—indicating that most of the patients did not have significant hormonal imbalances contributing to their infertility.

Table 3: Testicular Abnormalities in Male Infertile Patients (Ultrasonogram Findings)

Abnormality	Number of Patients (n = 64)	Percentage (%)
Epididymal Cyst	2	3.1
Bilateral Small Testes	4	6.25
Hypoplastic Testes	2	3.1
Testes with Microcalcification	2	3.1
Total with abnormalities	8	12.5

Table 3 presents the ultrasonogram findings for testicular abnormalities in male infertile patients. Out of 64 patients, 12.5% (4 patients) exhibited testicular abnormalities. These included bilateral small testes (6.25%), epididymal cyst (3.1%), hypoplastic testes (3.1%), and testes with microcalcification (3.1%). This suggests that, patients with structural testicular abnormalities may contribute to their infertility.

Table 4: Antichlamydia Antibody in Male Infertile Patients

Test Result	Number of Patients (n = 64)	Percentage (%)
Positive	10	15.63
Negative	54	84.38

Table 4 shows the results of the antichlamydia antibody test for male infertile patients. Out of 64 patients, 15.63% (10 patients) tested positive for the antibody, while 84.38% (54 patients) tested negative. This indicates that, patients with Chlamydia infection potentially be linked to infertility.

Table 5: Karyotype Findings in Male Infertile Patients

Karyotype	Number of Patients (n =64)	Percentage (%)
Normal (46XY)	60	93.75
Abnormal (47XXY)	4	6.25

Table 5 presents the karyotype findings in male infertile patients. The majority of patients (93.75%) had a normal karyotype (46XY), while 6.25% exhibited an abnormal karyotype, specifically 47XXY, indicating Klinefelter syndrome. This suggests that chromosomal abnormalities, may contribute to infertility in patients.

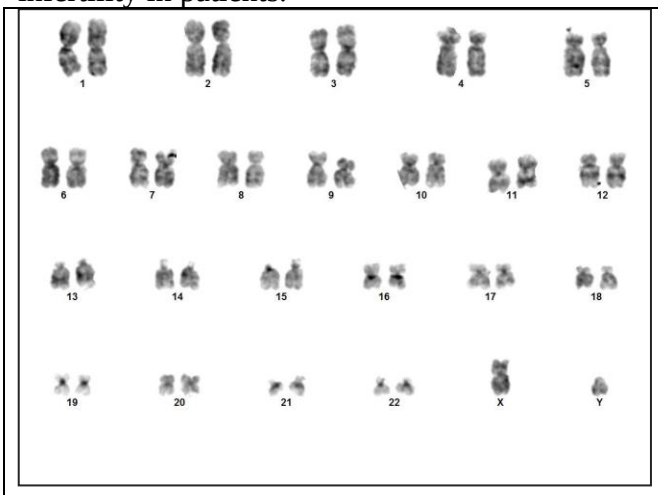

Figure 1: Karyogram showing a normal karyotype (46XY) from a Bangladeshi male infertile patient

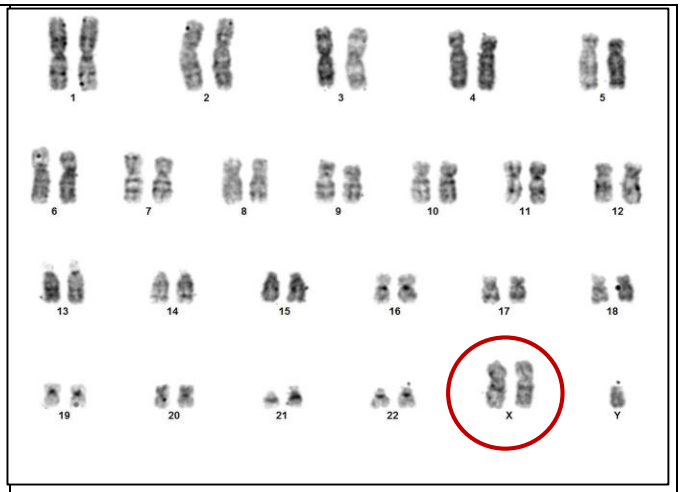

Figure 2: Karyogram showing an abnormal karyotype (47XXY) in a Bangladeshi male infertile patient indicative of Klinefelter syndrome

DISCUSSION

Male infertility is a major reproductive health issue globally, with genetic factors playing a crucial role in its etiology [15]. Karyotyping remains a primary diagnostic tool for detecting chromosomal abnormalities that

contribute to male infertility, particularly in azoospermic individuals. This study aimed to assess karyotypic abnormalities among selected Bangladeshi azoospermic male infertile patients, providing insights into the prevalence and clinical relevance of chromosomal variations in this population.

Age at diagnosis: The present study has been carried out on the 64 Bangladeshi male infertile patients whose mean age at diagnosis was 33.53 (± 6.37 SD) years, and the lowest age and the highest age were 22 years and 48 years respectively. About 56.3% of patients were within 31-40 years age group. One study conducted in Bangladesh represented that, about 63% of the patients presented to infertility clinic at their early middle age (30-40 years age range), that is consistent with this study outcome. The mean age at presentation is 35.5 years, that is also nearly consistent with present study result [4].

A secondary data analysis of the Avon Longitudinal Study of Pregnancy and Childhood which was a large population-based study in the United Kingdom. That survey observed 8559 pregnancies to determine the effect of age on time to pregnancy. After adjusting for female age, conception during a 12-month period was 30% less likely for men over age 40 years in comparison with men younger than age 30 years [16].

Body Mass Index (BMI): In this study, among 64 male infertile patients, 34 (53.13%) patients were in normal weight having had the BMI in the range of 18.5-25 kg/m2., 26 (40.63%) patients were overweight having had the BMI in the range of 25-30 kg/m2 and only 4 (6.25%) patients were obese having had the BMI >30. No underweight patients were found. A Bangladeshi study reported that, over half (50.8%) of the male infertile patients were overweight or obese which does not correspond with the present research [4]. Obesity alters the hypothalamic–pituitary–gonadal axis both centrally and peripherally, which result in hypogonadotropic, hyperestrogenic hypogonadism. Leptin and adipokines, which derived from adipose tissue regulate testosterone production and inflammation, respectively. Increased systemic inflammation results in increased reactive oxygen species and sperm DNA fragmentation [17]. Obesity is known to adversely affect male infertility through alteration in semen parameters (including sperm concentration, motility, viability, morphology, DNA integrity and mitochondrial function), endocrine changes (including hypogonadism, hyperinsuinemia and hyperleptinemia) and systemic and reproductive system inflammation and oxidative stress [18].

Education: In the present study, among 64 male infertile patients, the frequencies of infertility were different among the different educational level. The majority 59.38% (38) of the patients' education level was secondary school level. In a study conducted on Bangladeshi population found that ,over one-quarter (27.8%) of the patients had masters level education followed by 22.6% HSC, 21.7% SSC and 11.9% primary and 11.1% graduate level educated that is not consistent with the present research [4].

Occupation: In the present study the frequencies of infertility were different among different occupation . The incidence of infertility seems to be higher among service holder that is about 40.63% than others. Businessman were 37.50% patients and worker (garments factory worker, rickshawpuller, day labourer) were 21.88% patients and among 21.88% patients who were worker (garments factory worker, rickshawpuller, day labourer), there were about 6.25% patients were nickel factory worker. In terms of occupation, a study conducted on Bangladeshi population stated that, over one-third patients (34.5%) were private service-holder and another one-third (35.8%) was businessman and 12.4% govt.

service-holder that is nearly consistent with the present research [4].

Exposure history: The present research found 12.50% (08) male infertile patients had positive exposure history of multiple sexual partners. Multiple sexual partners is one of the causes which can affect the fertility of men [19]. Bangladeshi research represented that, majority (92.5%) of study population did not have any history of adverse environmental exposure. Only 4.4% reported occupational exposure to high temperature, 1.3% to pesticide, 0.9% to radiation and another 0.9% to chemical etc [4].

Family history of infertility: The information about the family history of infertility was obtained from the patient about paternal and maternal side. Only 04 (6.25%) patients had positive family history of infertility. Two patients had the history of infertility from paternal side and another two patients had the history of infertility from maternal side. One study reported the positive family history of infertility 17% that is not consistent with the present research [20]. Another study represented the positive family history of infertility among male infertile patients about 11.1% [21].

Hormone profile: The present study found 93.8% and 6.3% patients had respectively normal and abnormal level of serum testosterone, serum FSH and serum LH . The profile of serum TSH and serum prolactin was different, about 96.9% patients had within the normal range and only 3.1% patients had abnormal value. Studies have shown that blood testosterone level decline with the age and the risk of becoming infertile doubled in men who are over 35 years compared with men who are under 25 years old and five times longer to conceive at the age of 45 [22]. Production of testosterone hormone begins to decrease around the age of 40, sperm quality changes with aging, also there is decrease in the semen volume, motility and normal morphology [23]. Serum FSH and LH levels of patients with a genetic problem was significantly higher than in patients with no genetic problem ($P < 0.001$). Serum testosterone levels of patients with a genetic problem were significantly lower than in patients with no genetic problem ($P = 0.004$) [20]. Male reproductive hormones are produced and regulated by the hypothalamus-pituitary-endocrine system, which is responsible for stimulating the testicles to produce and release healthy sperm. In a small percentage of cases, only 1 to 2 percent, male infertility is due to problems in the hypothalamus and pituitary gland (parts of the brain that makes and regulates luteinizing hormone (LH) and follicle-stimulating hormone (FSH) cause the testes to produce testosterone and sperm). Thus, problems with the hypothalamus and pituitary glands can affect sperm production and sexual function [24].

Ultrasonogram findings: Additionally, 12.5% of patients exhibited testicular abnormalities on ultrasonography, including bilateral small testes (6.25%) and epididymal cysts (3.1%), which may contribute to spermatogenic dysfunction. Structural abnormalities in the testes, such as germ cell aplasia and seminiferous tubule fibrosis, have been reported in chromosomally abnormal men, particularly those with Y-chromosome deletions or rearrangements [12]. These findings emphasize the role of ultrasonography as a valuable non-invasive diagnostic tool in assessing azoospermic men with suspected genetic abnormalities. A study conducted in Bangladesh stated that, 11% of the respondents gave the history of trauma to the testes, 14.2% had history of suffering from varicocele that is consistent in terms of testicular abnormality but the type of abnormality is different with the present research [4]. Another study conducted in Saudi Arabia represented that, 40/74 patients had a varicocele [25].

Antichlamydia Antibody: The present research found 15.63% (10) patients had positive antichlamydia antibody out of 64 male infertile patients. This study found that 15.63% of participants tested positive for antichlamydia antibodies, suggesting prior or ongoing Chlamydia trachomatis infections. Chlamydia is a well-known cause of male infertility, contributing to epididymitis, seminal tract obstruction, and sperm DNA fragmentation [26] [27]. More than 15% had sexually transmitted diseases [4]. The "African Infertility Belt" also has high rates of STDs such as N. gonnorrhoeae and C. trachomatis, which may have some correlation and relationship with the high rates of infertility in this region of the world [26]. The relationship of chlamydia infection with male infertility is controversial [27]. A weak relationship of chlamydia infection with semen abnormality was observed in a study [28]. However, the relatively low prevalence of infection-related infertility in this cohort suggests that genetic factors may be the predominant contributors to azoospermia in Bangladeshi patients. These findings align with previous studies showing that while infections can impair male fertility, chromosomal abnormalities and Y-chromosome microdeletions are more frequently linked to non-obstructive azoospermia [8].

Karyotype findings: The study identified Klinefelter syndrome (47,XXY) in 6.25% of patients, while 93.75% exhibited a normal karyotype (46,XY). Klinefelter syndrome is the most common sex chromosomal disorder linked to male infertility, affecting 3–11% of azoospermic men worldwide [29]. It is characterized by testicular atrophy, depletion of germ cells, and hypergonadotropic hypogonadism, leading to primary testicular failure and impaired spermatogenesis [30]. The prevalence of Klinefelter syndrome in this study aligns with previous reports indicating that 5–10% of non-obstructive azoospermia cases are associated with this chromosomal anomaly [6,31]. A study stated that, the rate of chromosomal abnormality is 4.15% and the rate of chromosomal abnormality is highest in azoospermic patient (6.74%) in which numerical abnormality of sex chromosome was dominant mainly with Klinefelter's syndrome (KS) that is consistent with this study [6]. Another study represented; 14% infertile men had a genetic abnormality of which 7.2% cases were due to a karyotype abnormality that is also consistent with the present research. Of these karyotype abnormalities 71% due to Klinefelter's syndrome, 12% were translocations, 8% mixed gonadal dysgenesis, 4% XX male, and 4% 46XYY. Klinefelter's syndrome was detected in 8.7% of azoospermic males but there were none in the oligozoospermic group that is nearly consistent with the present research [20]. Klinefelter's syndrome have been reported as the most common chromosomal abnormality that is also consistent with this study [30]. Some other researches reported that, the most widely considered genetic factors are karyotype abnormalities that is 17% that is not consistent with the present research [32,33,34]. Revel et al. reported that 8.2% of infertile men had numerical and structural chromosomal anomalies, which was 20 times higher than rates in healthy fertile men that is nearly consistent with the present study [31]. The most common numeric abnormality in NOA is KS, which has an aberrant X chromosome, represented as 47,XXY, and is observed in one per 660 births, with up to 10%–12% occurring in NOA [29].

Kate et al. (2014) represented the chromosomal abnormality among azoospermic patient in India is about (2.02 %) that is not consistent with the present research [35]. Another research conducted in Central China reported the chromosomal abnormality about 1.36% [36] that is also not consistent with the present research. Chromosomal abnormalities

on karyotype analysis was found about 5% among infertile male that is nearly consistent with the present study [37]. Another study conducted in Western Saudi Arabia observed the chromosomal abnormality about 6.8% that is consistent with the present research [38].

Globally, chromosomal abnormalities have been reported in 4–15% of infertile men, with structural chromosomal aberrations such as translocations, inversions, and deletions also contributing to spermatogenic failure [39]. Although this study did not identify additional structural chromosomal abnormalities, the detection of Klinefelter syndrome underscores the need for routine karyotyping in azoospermic men, particularly those with unexplained infertility.

Given that 93.75% of patients exhibited a normal karyotype, further genetic testing, including Y-chromosome microdeletion analysis and whole-exome sequencing, is warranted for an in-depth assessment of azoospermic cases. Microdeletions in the AZF region (AZFa, AZFb, and AZFc) are well-documented genetic causes of spermatogenic failure [39]. Polymerase chain reaction (PCR) and gene sequencing techniques provide greater sensitivity for detecting these genetic defects, which may remain undetected by conventional karyotyping [10].

This study provides valuable insights into karyotypic abnormalities among Bangladeshi azoospermic men; however, certain limitations must be considered. The small sample size (n=64) limits the generalizability of the findings. Additionally, this study focused solely on numerical chromosomal abnormalities, without investigating structural aberrations or Y-chromosome microdeletions. The lack of molecular diagnostic techniques, such as aCGH or FISH, may have resulted in an underestimation of chromosomal variations. Furthermore, hormonal and testicular ultrasound findings were not extensively correlated with karyotypic results, which could provide additional clinical insights.

CONCLUSION

This study underscores the importance of karyotyping in diagnosing chromosomal abnormalities in Bangladeshi azoospermic men. The detection of Klinefelter syndrome (6.25%) aligns with global prevalence data, emphasizing the necessity of chromosomal analysis in routine infertility evaluations. Given that 93.75% of patients exhibited a normal karyotype, further genetic screening, including Y-chromosome microdeletion analysis and whole-genome sequencing, is recommended to identify additional genetic causes of azoospermia. Integrating karyotyping, molecular diagnostics, and genetic counseling into standard infertility care can improve clinical decision-making and reproductive outcomes for affected individuals.

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