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# AZF microdeletion affects semen parameters, sex hormone levels, and chromosome karyotypes in infertile men in Xinjiang

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**ABSTRACT**

This study aimed to analyze the correlation between the microdeletion of different regions of the azoospermia factor (AZF) gene and semen parameters, sex hormone levels, and karyotypes in infertile males by retrospective study. This was performed to obtain a comprehensive understanding of the clinical data of AZF microdeletion in infertile males, to guide clinical diagnoses and treatments, and to improve the efficacy and safety of assisted reproductive technology. For this purpose, Fifty-seven patients with AZF microdeletions and complete data were selected from 1916 patients with AZF microdeletions in our hospital from January 2020 to August 2022. The correlation between semen parameters, sex hormone levels, and chromosome karyotypes of these 57 patients was analyzed. Results showed that among the 57 patients with AZF microdeletions, the region with the highest microdeletion rate was AZFc with 57.89%; single or combined deletions in AZFa and AZFb regions resulted in azoospermia. The deletion frequency of AZFc in the oligospermia group was significantly higher than that in the azoospermia group, and the deletion frequencies of AZFb and AZFb + c in the azoospermia group were significantly higher than those in the oligospermia group ($P$<0.05). There were statistically significant differences in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, and chromosome karyotypes between patients with azoospermia and oligospermia ($P$<0.05). Statistically significant differences were observed in prolactin (PRL), FSH, testosterone (T), LH levels, and chromosome karyotypes of patients in different AZF microdeletion regions ($P$<0.05). In conclusion, AZF microdeletions can lead to a decline in semen quality in men, and different types of deletions have different effects on semen parameters, sex hormone levels, and karyotype analysis. Further treatments should be selected based on the AZF microdeletion area.

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## Introduction

Infertility is defined as the absence of conception one year after regular sexual intercourse without contraception. Male infertility is one of the main causes of infertility in couples of childbearing age. Approximately 10-15% of couples of childbearing age cannot have children, among which male factors account for 30–50% (1-3). Spermatogenesis disorders caused by genetic defects account for approximately 30% of male infertility (4). Chromosome karyotype abnormalities and Y-chromosome long-arm microdeletions are the most important genetic factors of male infertility (5). Chromosome number and structural abnormalities may lead to abnormalities in spermatogenesis. Azoospermia factor (AZF) microdeletion is mainly caused by the deletion of AZF on the Y chromosome. In 1976, Tiepolo et al. first proposed that AZF microdeletion was closely related to male infertility. Since then, the relationship between the AZF region and male infertility has become a key research focus (6). One study recommended AZFa, AZFb, and AZFc as three independent detection regions (7). AZF microdeletion can lead to non-obstructive azoospermia (NOA) and severe oligozoospermia, which then causes male infertility. Approximately 7-10% of severe oligozoospermia cases were caused by AZF microdeletions, while the proportion in NOA increased to 15-20% (8). The detection of AZF microdeletions has become an important diagnostic method for genetic male infertility, which is of great significance for guiding clinical treatment, improving the efficacy and safety of assisted reproductive technology, and carrying out preimplantation genetic diagnosis (9).

In this study, 57 patients with AZF microdeletions were selected to analyze the correlation between different regional deletions and semen parameters, sex hormone levels, and chromosome karyotypes, to obtain a more comprehensive understanding of the clinical data of male infertility with AZF microdeletions. This will provide a more comprehensive basis for clinicians to detect Y-chromosome microdeletions in male patients with infertility, help predict the prognosis of infertile patients with assisted reproductive technology and selected treatment methods, avoid unnecessary medical operations, and save medical costs.

## Materials and Methods

### Data collection

A total of 1916 infertile patients who came to our hospital from January 2020 to August 2022 were diagnosed with AZF microdeletion. Of these patients, 57 patients

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had complete data and were included in this study. The correlation between the type of AZF microdeletion region in these 57 patients (24-39 years of age) and their semen parameters, sex hormone levels, and chromosome karyotype analysis results were retrospectively analyzed.

**AZF microdeletion detection**

DNA was extracted using the blood gene column small quantity extraction kit according to the manufacturer's instructions and stored at -20 °C. (Shanghai Toujing Life Science and Technology Co. Ltd.) Microdeletions of six sequence tag loci (STS) in the AZFa (sY84, sY86), AZFb (sY127, sY134), and AZFc (sY254, sY255) regions were detected using two-tube multiplex PCR and four-channel fluorescence. Two internal control genes were included, the male sex determination gene (SRY) and the zinc finger protein coding gene (ZFY).

The PCR amplification cycling conditions were as follows: 50 °C for 2 min; 95 °C for 5 min; 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s, for 38 cycles; and 72 °C for 5 min. The FAM/VIC/ROX/Cy5 signal was recorded at 60 °C during the 38-cycle repeat. The experiment was considered successful if the FAM channel results exhibited typical S-shaped amplification curves and CT < 32.

**Semen parameter analysis**

Semen samples were collected by masturbation in sterile containers after 2-7 days of abstinence and immediately liquefied at 37 °C. Semen parameters were analyzed using the BEION S6 sperm quality analyzer (Shanghai Beiang Medical Technology Co., Ltd.), according to the diagnostic criteria of the World Health Organization(WHO) Laboratory Manual for Human Semen Examination and Processing (5th edition) (10). The following WHO semen analysis criteria were applied: sperm concentration was determined three times; if no sperm was observed in the fresh semen microscopic examination, but sperm was observed after centrifugation (3000 × g for 15 min), then this was called occult sperm disease. Samples were determined to exhibit asthenospermia if they had a sperm concentration of $< 15 \times 10^6$ cells/mL and progressive (PR) motility percentages of < 32%. The percentage of normal sperm was < 4% in asthenospermia semen samples. Normal semen samples exhibited sperm concentrations of $\geq 15 \times 10^6$ cells/mL, PR sperm percentages of ≥ 32%, and normal morphology sperm percentages of ≥ 4%.

**Chromosome karyotype analysis**

Peripheral blood (2-3 mL) was collected for heparin anticoagulation. Sterile inoculation of 0.5 mL anticoagulant blood in human peripheral blood lymphocyte culture medium (Guangzhou Dahui Biotechnology Co., Ltd.) was performed and placed in a 37 °C saturated humidity incubator for 68-72 h. Before the termination of culture, four drops of colchicine (20 μg/mL) were added to the medium, resulting in a final concentration of 0.04-0.08 μg/mL. The cells were harvested after continuous culture at 37 °C for 2.5 h. Chromosome preparations and G-banding karyotype analyses were performed; 20 metaphases were analyzed for each of the five chromosome karyotypes.

**Sex hormone detection**

Fasting venous blood samples were collected from the patients, centrifuged (3000 r/m, 10 min), and serum collected. Prolactin (PRL), follicle-stimulating hormone (FSH), testosterone (T), luteinizing hormone (LH), and estradiol (E2) levels were measured using an American Abbott i2000 chemiluminescence analyzer and the supporting reagents.

**Statistical analysis**

SPSS 26.0 software was used to perform the statistical analyses. Results are expressed as mean ± standard deviation or constituent ratio. Continuous variables were compared between the two groups using the t-test. Categorical variables were compared using the $\chi 2$ or Fisher's exact tests. Comparisons between multiple groups were performed using variance analysis. Statistical significance was set at $P<0.05$.

## Results

**Hormone levels and karyotypes of patients with microdeletions in different AZF regions**

In this study, 57 infertile male patients with AZF microdeletions were divided into azoospermia (n = 32) and oligospermia (n = 25) groups according to routine semen analysis. The average age of azoospermia patients was 30.22 ± 3.40 years, and the average age of oligozoospermia patients was 30.48 ± 3.18 years. There was no significant difference in age between the two groups ($P>0.05$). In the azoospermia group, the deletion rate of the AZFb + c region was the highest with 13 patients (40.6%). The frequency of other types of deletions was eight cases of AZFc region deletion (25.0%), five cases of AZFb region deletion (15.6%), three cases of AZFa + b + c region deletion (9.4%), two cases of AZFa region complete deletion (6.3%), and one case of AZFa region partial deletion (only SY86 deletion) (3.1%). AZF microdeletion occurred in the AZFc region in the oligospermic group, with 25 cases (100.0%). There were statistically significant differences in the deletion of the AZFb, AZFc, and AZFb + c regions ($P<0.05$).

The FSH levels in azoospermia and oligozoospermia cases were 15.15 ± 10.39 and 8.82 ± 2.92 IU/L, respectively, and the LH levels were 6.30 ± 4.23 and 3.91 ± 1.56 IU/L, respectively. These hormone levels were significantly different between the two groups ($P<0.05$). In the azoospermia group, 19 patients (59.4%) had normal sex chromosomes (46, XY), seven (21.9 %) had Y-chromosome long arm deletion, two (6.3 %) had y chromosome number abnormalities with long arm deletion, and four (12.5 %) had sex reversal (46, XX). In the oligozoospermia group, 24 patients (96.0%) had normal sex chromosomes (46, XY) and one (4%) had a Y-chromosome long arm deletion. There was a significant difference in chromosome karyotypes between azoospermia and oligospermia patients ($P<0.05$, Table 1).

**Hormone levels of infertile males with microdeletions in different AZF regions**

The hormone levels of 57 infertile males with microdeletions in different regions of AZF were compared, and the differences in PRL, FSH, T, and LH levels were significantly different ($P<0.05$, Table 2).

**Table 1.** Chromosome karyotype difference between azoospermia and oligospermia patients.

| | Azoospermia (n=32) | Oligozoospermia (n=25) | $\chi 2/t$ | *P* |
|---|---|---|---|---|
| **Age (year)** | 30.22±3.40 | 30.48±3.18 | -0.296 | 0.768 |
| **Y-gene deletion site** | | | | |
| AZFa | 2(6.3) | 0(0.0) | 2.474 | 0.499 |
| Part of AZFa | 1(3.1) | 0(0.0) | | |
| AZFb | 5(15.6) | 0(0.0) | 4.282 | 0.039 |
| AZFc | 8(25.0) | 25(100.0) | 33.287 | <0.001 |
| AZFb+c | 13(40.6) | 0(0.0) | 13.157 | <0.001 |
| AZFa+b+c | 3(9.4) | 0(0.0) | 2.474 | 0.116 |
| **Hormone levels** | | | | |
| PRL (mIU/L) | 234.36±210.92 | 188.60±99.95 | 0.999 | 0.322 |
| FSH (IU/L) | 15.15±10.39 | 8.82±2.92 | 2.952 | 0.002 |
| T (nIU/L) | 13.12±3.87 | 11.99±3.56 | 1.130 | 0.264 |
| LH (IU/L) | 6.30±4.23 | 3.91±1.56 | 2.677 | 0.010 |
| $E_2$ (pmol/L) | 79.16±29.88 | 87.12±29.20 | -1.007 | 0.318 |
| **Chromosomes** | | | 10.683 | 0.014 |
| Normal | 19(59.4) | 24(96.0) | | |
| Y-chromosome deletion | 7(21.9) | 1(4.0) | | |
| Chimerism/structural anomalies | 2(6.3) | 0(0.0) | | |
| Sexual reversal | 4(12.5) | 0(0.0) | | |

**Table 2.** Comparison of hormone levels in infertile men with microdeletions in different AZF regions.

| | N | PRL (mIU/L) | FSH (IU/L) | T (nIU/L) | LH (IU/L) | $E_2$ (pmol/L) |
|---|---|---|---|---|---|---|
| only AZFa | 2 | 140.09±45.65 | 16.29±2.64 | 18.89±5.13 | 5.76±2.55 | 70.00±12.73 |
| only part of AZFa | 1 | 1143.54 | 6.76 | 12.78 | 3.43 | 55.00 |
| only AZFb | 5 | 184.91±78.58 | 7.58±3.59 | 14.32±2.06 | 4.18±2.01 | 78.80±28.29 |
| only AZFc | 33 | 210.67±126.79 | 9.53±4.46 | 12.78±3.72 | 3.95±1.50 | 85.04±30.00 |
| AZFb+c | 13 | 174.01±131.06 | 15.53±8.50 | 11.81±3.08 | 6.66±3.91 | 84.00±35.56 |
| AZFa+b+c | 3 | 217.34±16.70 | 37.56±6.48 | 7.38±0.90 | 15.53±1.81 | 74.67±8.08 |
| *P* | 57 | <0.001 | <0.001 | 0.015 | <0.001 | 0.889 |

**Table 3.** Karyotype differences in infertile males with microdeletion in different AZF regions.

| | Normal | Y- chromosome deletion | Chimerism/structural anomalies | Sexual reversal | P |
|---|---|---|---|---|---|
| only AZFa | 1 | 1 | 0 | 0 | <0.001 |
| only part of AZFa | 1 | 0 | 0 | 0 | |
| only AZFb | 5 | 0 | 0 | 0 | |
| only AZFc | 32 | 1 | 0 | 0 | |
| AZFb+c | 4 | 6 | 2 | 1 | |
| AZFa+b+c | 0 | 0 | 0 | 3 | |

**Karyotype analysis of infertile males with microdeletions in different AZF regions**

The karyotypes of 57 infertile males with microdeletions in different AZF regions were compared. A Fisher's exact test revealed significant differences in karyotypes of infertile men with microdeletions in different AZF regions ($P<0.05$, Table 3).

## Discussion

Primary male azoospermia and severe oligospermia are among the main causes of male infertility and have attracted increasing attention in clinical practice. The detection of AZF microdeletions has become an important diagnostic method for determining hereditary male infertility (11-13). This can help the clinical diagnosis of underlying azoospermia or severe oligospermia causes and has become a routine infertility test project in Europe and the United States (14,15). AZF is located in the Yq11 region of the Y chromosome. Many palindromic sequences are present on the Y chromosome. Internal non-allelic homologous recombination is the main cause of Y chromosome microdeletion (16). Some Y-chromosome homologous sequences are on the X or autosomal chromosomes; therefore, homologous recombination may lead to Y-chromosome microdeletions (17).

AZF microdeletion can be as a result of single-site, region, or several regions combined deletions. Male infertility caused by the deletion of six sequence tag loci (sY84, sY86, sY127, sY134, sY254, and sY255), recommended

by the European standard screening scheme, accounts for 95% of all AZF microdeletions (18). In this study, AZF microdeletions were detected in 1916 infertile patients who visited our hospital. Seventy-three cases of AZF microdeletions were detected, with an abnormality rate of 3.81%. The results were similar to those of 54 cases (3.3%) of AZF microdeletions detected in 1616 infertile patients by Akınsal et al. (19). This was significantly lower than those of 30 cases (58.8%) of AZF microdeletions detected in 51 infertile patients by Elsaid et al. (20). The frequency of AZF deletion in infertile patients ranges from 0.59 to 32.62% (average = 13.48%) (21).

AZFc microdeletion was the most common microdeletion observed among patients in the current study, with the highest occurrence rate of 33 cases (57.89%), which was lower than that reported by Bahmanimehr et al. (75%) (22). The second most common microdeletion in the current study was AZFb + c, with 13 cases (22.81%). The incidence of AZFa partial deletion (only SY86 deletion) was the least prevalent, with one case (1.75%). The frequency of AZF microdeletions in other studies differ and are caused by many factors. Some of these differences can be attributed to the following: (I) the density and location of the STS markers used for detection were different; (II) different detection methods were used; (III) the study populations exhibited different genetic backgrounds and environments; (IV) differences in specimen quantity; and (V) selection bias of the subjects (5).

In this study, 25 (75.76%) of the 33 patients with AZFc microdeletions exhibited oligospermia. Zhu et al. (5) reported that 85.29% of patients with AZFc microdeletions had sperm. Johnson et al. reported that only 27.3% of patients with AZFc microdeletions had sperm (23). Hopps et al. (24) and Oates et al. (25) reported that 38% of patients with AZFc microdeletions had sperm. Potential reasons for the differences in sperm detection rates can be attributed to the technical competency of laboratory personnel, group differences, and the sizes of the AZF microdeletion areas. This also reflects the diversity of clinical manifestations in patients with AZFc microdeletions. It can range from severe oligozoospermia to azoospermia. It can be used as a key screening tool for patients with azoospermia or severe oligozoospermia. This suggests that the degree of AZF microdeletion may be related to the degree of spermatogenesis disorder.

In this study, other AZF deletion types all resulted in azoospermia, and the deletion rate of the AZFb + c region was the highest, with 13 patients (40.6%), which was consistent with the results of Colaco et al. (26). AZFa microdeletion causes azoospermia and maintenance cell syndrome; this AZF region microdeletion in patients displayed the worst prognosis. The clinical manifestation of AZFb deficiency is spermatogenesis halted in the spermatocyte stage; therefore, spermatogonia and primary spermatocytes are still visible in the testis, but there is no spermatogenesis (27). Therefore, the single or combined deletion of the AZFa and AZFb regions is characterized by azoospermia.

FSH and LH levels in the azoospermic group were significantly higher than those in the oligospermic group ($P$<0.05). The levels of PRL, FSH, and LH in patients with the AZFa + b + c microdeletion were significantly higher than those with other microdeletions, and the level of T was significantly lower than that in other microdeletions ($P$<0.05). This suggests that different AZF deletion types were closely related to PRL, FSH, LH, and T sex hormones. These results are different from those reported by Elsaid et al. (20) and Mascarenhas et al. (28). Potential reasons for these differences are group differences, namely fewer cases included in the study, and a lack of group representation.

Although AZFc region deletion patients are able to produce sperm, the increase in FSH levels results in the number of sperm showing a progressive downward trend; therefore, early intervention treatment or cryopreservation after non-invasive sperm extraction is necessary (29). Previous studies have suggested that AZF microdeletions may affect spermatogenesis and hormone levels differently compared to other known male diseases. The relationship between AZF microdeletions and reproductive hormone levels requires further investigation.

In this study, 24 (96%) of 25 patients with oligospermia had normal sex chromosomes. Some patients could obtain sperm by testicular sperm extraction and other technologies, and produce theoretically biological offspring. Y chromosome abnormalities were found in 14 patients (24.56%), of which 13 patients (92.86%) were in the azoospermia group. The frequency of long arm deletion in Y chromosome abnormality was the highest, with eight cases (14.04%), which was consistent with the results of related studies (30). Four cases (7.02%) of sexual inversion (46, XX) and two cases (3.51%) with Y chromosome number abnormality and long arm deletion were observed in the azoospermia group. The karyotypes were 45,X[22]/46,X,del(Y) (q11)[93] and 45,X[32]/46,X,del(Y) (q11) [21], respectively. Patients with AZFc microdeletion had the highest probability of normal chromosomes, with 32 cases (56.14%), and patients with AZFb + c microdeletion had the highest probability of Y chromosome abnormality, with 9 cases (15.79%). Y chromosome abnormalities were not detected in some AZFa (only SY86) or AZFb deletions.

Chromosomal aberration is an important factor in male infertility. This study showed that the incidence of chromosomal abnormalities was inversely proportional to spermatogenesis, and the incidence of sexual chromosomal abnormalities in infertile males was higher than that in fertile males (31). If the Yq11 region is absent, AZF should not be detected. However, traditional chromosome karyotype analysis can only detect deletions greater than 10 Mb. The sizes of the AZFa, b, and c regions are between 792 kb and 3.5 Mb, and even the combined deletions of the AZFb and c regions are less than 7.7 Mb. Therefore, chromosome karyotype analysis cannot replace AZF gene detection.

In summary, AZF microdeletion may be an important factor that affects NOA. AZF microdeletion in infertile patients is closely related to patient hormone levels and chromosome karyotypes. A joint analysis has important theoretical and clinical significance. It can provide a theoretical basis for genetic counseling in assisted reproductive therapy and a scientific basis for the diagnosis and treatment of male infertility. In males with microdeletions, AZF microdeletion screening can be used to predict the chance of sperm retrieval when men accept testicular sperm extraction (TESE) if they consider using assisted reproductive technology to produce the next generation. Patients with AZFc microdeletion have the opportunity to have child-

ren by extracting sperm from TESE. Additionally, it is recommended to avoid unnecessary surgery and medical treatment in cases of AZFa and AZFb microdeletion. In future research, the sample size should be increased, and the impact of regional differences should be considered to further improve the rigor of the research results.

**Data availability**

The data that support the findings of this study are available on request from the corresponding author.

**Conflicts of interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Ultrastructure and pathogenic genes of multiple malformations of sperm flagella in infertile men in Xinjiang.

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