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Male Infertility

# Success Rate of Microsurgical Multiple Testicular Sperm Extraction and Sperm Presence in the Ejaculate in Korean Men With Y Chromosome Microdeletions

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**Purpose:** We assessed the frequency of azoospermia factor a (AZFa), AZFb, and AZFc deletions and examined correlations between the deletion sites and the success rates of sperm presence within the ejaculate and surgical sperm retrieval in Korean men.
**Materials and Methods:** A total of 1,919 azoospermic and severely oligozoospermic men were assessed for Y chromosome microdeletions. Among them, 168 men with AZF deletions were identified and their medical records were reviewed.
**Results:** Of the total 168 men with AZF deletions, there were 13 with AZFa, 10 with AZFb, 95 with AZFc, 37 with AZFbc, and 13 with AZFabc deletions. Of the 95 men with isolated AZFc deletion, 51 had the presence of sperm in the ejaculate. Of the infertile men with any other deletion, however, only two patients (one man with AZFb deletion and another with AZFbc deletion) showed the presence of sperm in the ejaculate. The success rates for surgical sperm retrieval were 7.1% (1/14) in men with AZFbc deletion and 54.8% (17/31) in the isolated AZFc deletion group. No sperm was obtained from the patients with AZFa or AZFb deletions who underwent microsurgical sperm retrieval. In the isolated AZFc deletion group, there were significant differences between azoospermic and severely oligozoospermic patients in terms of testicular volume and serum levels of follicle-stimulating hormone and luteinizing hormone, whereas no significant differences were found when the group was divided by surgical sperm retrieval outcomes.
**Conclusions:** Deletions of the AZFa and AZFb regions are associated with severe spermatogenetic impairment. However, more than half of men with an AZFc deletion had sperm within the ejaculate or testis for in vitro fertilization with intracytoplasmic sperm injection.

***Keywords:*** *Azoospermia; Infertility; Male; Y chromosome*



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## INTRODUCTION

Approximately 15% of couples attempting to conceive over the course of a year are infertile, and approximately half of these cases of infertility are attributable to a male factor [1,2]. Several known causes of male infertility have been described [3]; however, up to 40% of male cases of infertility are of unknown etiology [4]. Genetic problems are considered to be an important factor in male infertility, and up to 12% of men with nonobstructive azoospermia have karyotype abnormalities [5].

The Y chromosome plays an important role not only for sex determination but also for normal spermatogenesis. Microdeletions of the long arm of the Y chromosome (Yq) are known to represent a pathogenic mechanism in males with azoospermia or oligozoospermia and are the second most frequent gene-level cause of spermatogenic failure after Klinefelter syndrome [6-9]. In the Y chromosome, the

specific genes involved in the complex process of spermatogenesis are located on the distal Yq11 region and are defined as the azoospermia factor (AZF).

The most common Y chromosome microdeletions occur in the AZFc region, representing approximately 60% of all microdeletions [10]. Previous studies have demonstrated that the specific location of the microdeletion within AZF determines the specific effects on semen parameters, which range from oligozoospermia to azoospermia and on testis histological findings such as Sertoli cell only, maturation arrest, and hypospermatogenesis [11,12]. Many earlier studies revealed that the identification of sperm in the ejaculate and the surgical retrieval of sperm are most successful in men with deletion of the AZFc region and are rarely successful when the deletions are in the AZFa or AZFb regions [13-15].

The present study aimed to assess the prevalence of specific AZFa, AZFb, AZFc, and combined-region deletions in a large number of infertile men in Korea and to evaluate the prognosis of each type of deletion on the basis of the potential for identifying sperm within the ejaculate or obtaining sperm by surgical retrieval for *in vitro* fertilization with intracytoplasmic sperm injection.

## MATERIALS AND METHODS

A total of 1,919 patients with severe oligozoospermia ($< 10 \times 10^6$ sperm/mL) or azoospermia were screened for AZF deletion between September 1997 and June 2012. Informed consent was obtained from all patients who underwent Y chromosome microdeletion analysis. Among them, a total of 168 patients were found to have an AZF deletion at one or more sites and were included in this study. Medical records were retrospectively reviewed to determine the patient's age, semen analysis results according to the guidelines of the World Health Organization, results of testis biopsy, hormonal profile (testosterone, follicle-stimulating hormone [FSH], and luteinizing hormone [LH]), testicular volume, chromosome analysis, and the results of microsurgical multiple testicular sperm extraction (m-TESE). Among the 95 patients identified as having AZFc deletion, 61 patients had testis biopsies reported and 31 patients (21 with azoospermia and 10 with severe oligospermia) had undergone surgical sperm retrieval through microsurgical m-TESE. The number of patients with AZFa, AZFb, and AZFbc deletions who underwent microsurgical m-TESE were 5, 8, and 14, respectively. None of AZFabc-deleted patients underwent surgical sperm retrieval. We performed surgical retrieval on the day of *in vitro* fertilization with intracytoplasmic sperm injection when the patient's semen specimen obtained immediately before m-TESE showed azoospermia although the patient had shown the presence of sperm in the previous semen analysis.

For analysis of Y chromosome microdeletions, genomic DNA was extracted from a peripheral venous blood sample from each patient, and sequence-tagged site (STS) markers specific for the Y chromosome were used. From September 1997 to April 2009, the STS markers used in our laboratory were sY14 (an *SY* gene used as an internal control), sY84 (AZFa region), sY129 and sY134 (AZFb region), and sY254 and sY255 (within the *DAZ* gene, AZFc region). Starting from May 2009, we added additional AZF site polymerase chain reaction (PCR) amplification primers for the analysis of Y chromosome microdeletions. Those included sY86 for AZFa; sY124, sY127, and sY130 for AZFb; and sY147, sY157, sY158, sY242, and SPGY1 for AZFc. PCR was performed in a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Hormonal profiles included serum testosterone, FSH, and LH levels as assessed by radioimmunoassay and were determined to be within the normal range at 1.3–8.13 ng/mL, 1–14 mIU/mL, and 1.5–9.2 mIU/mL, respectively. Microsurgical m-TESE was performed through a single transverse incision in the tunica albuginea for extraction of spermatogenic tubules by operative microscopy.

For statistical analyses, IBM SPSS ver. 18.0 (IBM Co., Armonk, NY, USA) was used. Outcome variables, including age, serum testosterone, FSH, LH, and testicular volume for each AZF deletion group were compared by using the Kruskal-Wallis test. Differences in the above variables and histological results from testis biopsy between the isolated AZFc-deleted patients in which sperm was successfully obtained from ejaculate or surgical extraction and those in which retrieval was not successful were evaluated by using the Mann-Whitney U test. Statistical significance was defined as a p-value less than 0.05.

## RESULTS

AZF deletion was detected in 168 patients, giving a prevalence rate of 8.8% (168/1,919). The most commonly deleted region was AZFc (56.6%, 95/168), and deletions of the AZFa, AZFb, AZFbc, and AZFabc regions represented 7.7% (13/168), 6.0% (10/168), 22.0% (37/168), and 7.7% (13/168) of the sample, respectively (Table 1). Including combined AZF deletions, such as AZFbc and AZFabc, the overall prevalence of AZFc deletion was 86.3% (145/168).

Age and testosterone levels were not significantly different between the different AZF deletion groups. However, the AZFb deletion group had a normal mean testis volume, a normal FSH level, and a lower LH level than did the other AZF deletion groups, resulting in significant differences between this group and the other groups for these measurements (Table 1).

All patients with AZFa, AZFb, AZFbc, or AZFabc deletions were azoospermic, except for two patients (one each with AZFb and AZFbc deletions) who had severe oligozoospermia. In contrast, only 46.3% of patients (44/95) with isolated AZFc deletion were azoospermic, and 53.7% of the AZFc deletion patients (51/95) retained some level of spermatogenesis to produce sperm within the ejaculate (Table 2). For each one patient with the AZFb or AZFbc deletion

**TABLE 1.** Age, testis size, and hormonal profile of patients with microdeletions by deletion group

| Variable | AZFa (n=13) | AZFb (n=10) | AZFc (n=95) | AZFbc (n=37) | AZFabc (n=13) | p-value |
|---|---|---|---|---|---|---|
| Age (y) | 32.9±3.5 | 32.4±3.2 | 32.6±3.2 | 34.3±5.0 | 33.8±3.8 | 0.541 |
| Testis volume (mL) | | | | | | |
| Right[a] | 12.3±6.1 | 17.1±5.0 | 13.5±4.4 | 12.4±4.5 | 7.6±4.5 | <0.001 |
| Left[a] | 12.2±5.9 | 16.2±4.1 | 13.1±4.1 | 12.2±4.3 | 7.8±4.8 | <0.001 |
| Hormonal profile | | | | | | |
| FSH (mIU/mL)[a] | 24.1±14.4 | 7.9±3.8 | 15.9±9.6 | 21.9±14.1 | 22.2±14.5 | <0.001 |
| LH (mIU/mL)[a] | 7.8±6.7 | 5.0±2.1 | 5.2±3.4 | 7.7±3.4 | 8.7±7.3 | 0.001 |
| Testosterone (ng/mL) | 3.4±1.7 | 3.9±1.7 | 4.0±1.7 | 3.5±1.4 | 3.2±1.5 | 0.106 |

Values are presented as mean±standard deviation.
AZF, azoospermia factor; FSH, follicle-stimulating hormone; LH, luteinizing hormone.
[a]:Statistically different factor between groups.

**TABLE 2.** Success rates of surgical sperm retrieval and sperm presence within ejaculate by deletion group

| | AZFa | AZFb | AZFc | AZFbc | AZFabc | p-value |
|---|---|---|---|---|---|---|
| Sperm presence within ejaculate[a] | 0/13 (0) | 1/10 (10.0) | 51/95 (53.7) | 1/37 (2.7) | 0/13 (0) | <0.001 |
| Spermatozoa presence from surgical retrieval[a] | 0/5 (0) | 0/8 (0) | 17/31 (54.8) | 1/14 (7.1) | - | <0.001 |

Values are presented as frequency (%).
AZF, azoospermia factor.
[a]:Statistically different factor between groups.

**TABLE 3.** Associations between age, testis size, and hormonal profiles with absence or presence of sperm in the ejaculate and surgical retrieval, in the subset of patients with AZFc deletions

| Variable | Ejaculated sperm | | | Sperm from surgical retrieval | | |
|---|---|---|---|---|---|---|
| | Absent (n=44) | Present (n=51) | p-value | Absent (n=14) | Present (n=17) | p-value |
| Age (y) | 32.8±3.4 | 32.5±3.1 | 0.881 | 33.1±3.6 | 34.2±3.4 | 0.318 |
| Testis volume (mL) | | | | | | |
| Right | 12.0±4.7 | 14.8±3.7 | 0.001 | 13.1±4.7 | 14.2±3.5 | 0.236 |
| Left | 11.8±4.7 | 14.4±3.2 | 0.001 | 12.9±4.3 | 13.9±3.7 | 0.365 |
| Hormonal profile | | | | | | |
| FSH (mIU/mL) | 20.4±9.4 | 12.3±8.3 | <0.001 | 19.4±7.6 | 15.2±11.4 | 0.078 |
| LH (mIU/mL) | 6.4±4.0 | 4.1±2.2 | 0.003 | 6.4±3.9 | 5.3±2.2 | 0.642 |
| Testosterone (ng/mL) | 3.8±1.4 | 4.2±1.9 | 0.377 | 4.1±3.0 | 4.0±1.4 | 0.625 |

Values are presented as mean±standard deviation.
AZF, azoospermia factor; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

who had some sperm present within the ejaculate, the testis biopsy revealed hypospermatogenesis for the AZFb deletion patient and Sertoli cell only for the AZFbc deletion patient. The success rate for surgical retrieval of sperm by microsurgical m-TESE was 7.1% (1/14) in the AZFbc deletion group with maturation arrest histology and 54.8% (17/31) in the isolated AZFc deletion group. No sperm was obtained from the patients with AZFa or AZFb deletions who underwent microsurgical m-TESE. None of the 13 patients with AZFabc deletion elected to undergo surgical sperm retrieval (Table 2).

Age; serum levels of testosterone, FSH, and LH; and testicular volume were analyzed in the isolated AZFc deletion group. The comparison of men with AZFc deletions and either success or failure of surgical retrieval of sperm did not demonstrate any statistically significant differences. Azoospermic men with AZFc deletion had both significantly higher serum FSH and LH levels and lower testicular volumes than did severely oligozoospermic men with AZFc deletion. Age and serum testosterone levels in azoospermic men with the AZFc deletion were not significantly different from those in the severely oligospermic group (Table 3).

The histological findings of the testis biopsies are given in Table 4. The results showed that the presence of Sertoli cell only was highest in the group in which no sperm was

**TABLE 4.** Histological findings in patients with AZFc deletions by absence or presence of sperm in the ejaculate and through surgical retrieval

| | Ejaculated sperm (n=61) | | Sperm from surgical retrieval (n=31) | |
|---|---|---|---|---|
| | Absent | Present | Absent | Present |
| Testis biopsy | | | | |
| Normal | 0 | 1 | 0 | 1 |
| Sertoli cell only | 16 | 4 | 8 | 5 |
| Maturation arrest | 11 | 10 | 5 | 5 |
| Hypospermatogenesis | 5 | 14 | 1 | 6 |

Values are presented as absolute frequencies.
AZF, azoospermia factor.

identified either in the ejaculate or by surgical means and that hypospermatogenesis was the most common finding in the group in which sperm was present.

## DISCUSSION

Y chromosome microdeletion affecting spermatogenesis has been well established as a prevalent cause of male factor infertility [4,12,13,16-19]. On the Y chromosome, the specific genes involved in the complex process of spermatogenesis are located on the distal Yq11 region and are defined as the azoospermia factor. The AZF region has subregions termed AZFa, AZFb, and AZFc. The AZFa locus is located on proximal Yq11, whereas AZFb and AZFc are sequentially overlapping and located in the distal part of Yq11 [14]. In previous studies, Y chromosome microdeletion was detected in approximately 15% of azoospermic and 5% to 10% of oligozoospermic men. The frequency of microdeletions detected in our study was 8.8%, which was consistent with the range of 5.7% to 21.0% reported in other works [9]. As discussed earlier, this wide variation may be due to ethnic differences, patient selection factors, differences in genetic background, or the specific STS primers used [20]. We note in particular that our previous report of the frequency of Y chromosome microdeletion suggested a 7.7% (101/1309) prevalence [20] when we used the group of primers described in the Methods section. However, the detection of microdeletions increased with the addition of new primers for each AZF region, and the prevalence of microdeletions was 10.9% (67/613) between June 2009 and July 2012. The most frequent microdeletion found in our subjects was AZFc (56.6%) in the isolated form; moreover, when conjugated microdeletions (AZFbc and AZFabc) were included, the overall frequency of AZFc deletion increased to 85.3%. Isolated microdeletions of AZFa and AZFb were relatively rare.

Although we found that age, serum testosterone, FSH, LH, and testicular volume were significantly different across the microdeletion groups (Table 1), we believe that this significant result was largely driven by the fact that the mean hormones levels and testicular volumes of men with AZFb deletion were within the normal range compared with the higher hormone levels and smaller testicular volumes of men with other AZF deletions. We hypothesize that this is related to the fact that 7 of the 10 testis biopsies of patients with the AZFb deletion were histologically characterized as maturation arrest, which is defined as normal gonadotrophin levels and testicular volume (data not shown).

The isolated AZFc deletion group showed the highest rate of sperm presence within the ejaculate and had the highest rate of surgical retrieval success (53.7% and 54.8%, respectively; Table 2). These results are very consistent with the descriptions presented in previous studies, which showed that 38% of men with AZFc deletion had sperm in their ejaculate and that 56% to 67% had sustained some levels of spermatogenesis, allowing for successful extraction of spermatozoa [13,21]. An association between the detection of sperm within the ejaculate or surgical retrieval success and having an AZF deletion other than in the AZFc region has been suggested in several small cohort studies. One study documented that their entire sample of men with AZFa, AZFb, and conjugated forms of AZF deletions was azoospermic and that they failed to find sperm through either TESE or testis biopsy [21]. The proposed mechanism for this is that an autosomal copy of the DAZ gene (deleted in azoospermia in the AZFc region) located on chromosome 3, called DAZL (DAZ-like), may act as a "backup," which would help to preserve a small amount of residual spermatogenesis in males with AZFc deletions that remove the DAZ genes. In contrast, AZFa and AZFb have no such autosomal "backup" genes [13,22]. In our study, sperm was detected within the ejaculate for two patients who had either AZFb deletion or AZFbc deletion, although both were severely oligozoospermic. Surgical sperm retrieval in other cases except AZFc deletion was possible in only one patient with the AZFbc deletion.

Men with AZFc deletion have a variable capacity to produce sperm from either ejaculate or surgical extraction. We compared age, serum testosterone, FSH, LH, and testicular volume among AZFc-deleted men to identify factors associated with sperm presence and successful surgical extraction. As shown in Table 3, within the group of men with an AZFc deletion, identification of sperm within the ejaculate was correlated with serum FSH and LH and testicular volume independent of age and serum testosterone level. This result conflicts with previous studies that suggested that FSH and testicular volume reflect overall testicular function and cannot predict the presence of sperm in the ejaculate or successful sperm retrieval [13,18,21,23]. We do acknowledge, however, that none of the factors (age, serum testosterone, FSH and LH, or testicular volume) we evaluated was associated with successful surgical sperm retrieval. We conclude that neither mean age nor serum testosterone predicts either sperm presence within the ejaculate or successful surgical sperm retrieval, whereas serum FSH and LH and testicular volume predict the capacity to maintain spermatogenesis at a level sufficient to

spill sperm in the ejaculate.

In the 61 patients with AZFc deletion in whom a testis biopsy had been taken, as expected, hypospermatogenesis was the most prevalent histological finding in patients who either had sperm present in the ejaculate or in whom surgical retrieval was successful, and Sertoli cell only was the most common finding in AZFc patients who did not have sperm either in the ejaculate or on attempted surgical retrieval.

## CONCLUSIONS

This study describes the outcomes of microsurgical m-TESE and ejaculated sperm identification for infertile patients with Y chromosome microdeletions in Korea. Attempting to identify sperm within the ejaculate or through surgical sperm retrieval for *in vitro* fertilization with intracytoplasmic sperm injection is ineffective for males with AZFa and AZFb deletions. In contrast, more than half of men with isolated AZFc deletions can successfully have sperm surgically extracted for *in vitro* fertilization with intracytoplasmic sperm injection. Therefore, identification of the specific AZF deletion in infertile men with azoospermia or severe oligozoospermia is helpful to determine the etiology and probability of the presence of sperm before attempting surgical retrieval after proper genetic counseling regarding the vertical transmission of genetic abnormalities.

## CONFLICTS OF INTEREST

The authors have nothing to disclose.

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