

# Effects of a Partial Deletion of the Y Chromosome Azoospermia Factor (gr/gr Deletion) on Intracytoplasmic Sperm Injection Outcomes in East Asian Patients

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## ABSTRACT

**Introduction:** The azoospermia factor (AZF), located on the long arm of the Y chromosome, is involved in spermatogenesis. The gr/gr deletion, a partial deletion in the AZFc region, shows a high frequency in Asian men with infertility. Generally, gr/gr deletions do not strongly influence fertility in Asians; however, whether they affect intracytoplasmic sperm injection outcomes remains unclear. This study aimed to examine the effects of AZF deletions, particularly the gr/gr deletion, on intracytoplasmic sperm injection outcomes in East Asian subjects.

**Methods:** East Asian patients (204) with a normal male karyotype who underwent AZF testing between July 2014 and August 2018 were selected for this retrospective study (ethics committee approval number: A18024).

**Results:** Of the 204 East Asian subjects, 83 exhibited AZF deletions, of which 65 exhibited the gr/gr deletion. Thirty-seven patients in the gr/gr deletion group (56.9%) and seventy-seven in the group without AZF deletion (65.3%) were subjected to testicular sperm extraction (TESE), including microdissection-TESE (MD-TESE). Sperm retrieval was performed for 18 patients (48.6%) in the gr/gr deletion group and 36 patients (46.8%) in the group without AZF deletion. The fertilization rate, as ascertained using the TESE-derived sperms, was 54.8% (85/155) for the gr/gr deletion group and 66.5% (326/490) for the group without AZF deletion ( $p < 0.01$ ).

**Conclusions:** The fertilization rate was significantly lower for the sperm samples from the gr/gr deletion group than the control group. AZF gr/gr deletion did not affect spermatogenesis, but it may affect the fertilizing capacity of testicular sperms in East Asians.

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**KEYWORDS:** azoospermia factor, intracytoplasmic sperm injection, gr/gr deletion, testicular sperm extraction

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## Introduction

Y chromosome microdeletions are the second most common genetic cause of male infertility after Klinefelter syndrome.<sup>1)</sup> The prevalence of Y chromosome microdeletions in nonobstructive azoospermia (NOA) has been reported to be 2%-10%.<sup>2-4)</sup> Although NOA invariably leads to infertility, focal sperm production may exist in the testicles of affected patients, which can be retrieved and used for intracytoplasmic sperm injection (ICSI) to generate healthy offspring.<sup>5)</sup> The azoospermia factor (AZF), located on the long arm of the Y chromosome, is involved in spermatogenesis. The AZF region was discovered in 1976.<sup>6)</sup> Vogt *et al.*<sup>7)</sup> identified the three subregions of the AZF, namely, AZFa, AZFb, and AZFc. Testicular biopsies were performed to show the corresponding phenotypes of the deletion of each AZF subregion. Sertoli-cell-only syndrome was reported in AZFa deletion patients, mutation arrest was reported in AZFb-deletion patients, various histotypes were reported in AZFc deletion patients, and testes with a dysfunctional spermatogenesis were reported in AZFa- and AZFb-deletion patients. In AZFa- and AZFb-deletion patients, sperm retrieval was not possible even with testicular sperm extraction (TESE).<sup>6)</sup> According to the guidelines of the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN), TESE is not necessary in patients with complete AZFa, AZFb, or AZFb+c. Therefore, AZF tests are recommended for patients with azoospermia and severe oligospermia who wish to undergo ICSI and/or TESE.<sup>8)</sup> Furthermore, male infants who are conceived via ICSI can potentially inherit the genetic abnormalities of the paternal Y chromosome. Therefore, AZF tests and genetic counseling are important in establishing efficient approaches for treating infertility treatment.

In male patients with infertility, the frequency of retained AZF deletions is estimated to be 7% (95% confidence level [CL]: 6.74-6.79) worldwide.<sup>9)</sup> The frequency of microdeletions on the long arm of the Y chromosome is race- or ethnicity-dependent. The incidence of microdeletions is estimated to be 3% (95% CL: 2.9-3.0) in Europe, 5.3% (95% CL: 5.9-7.8) in Australia, and 8% in Asia.<sup>10)</sup> A categorization of AZF deletions can also yield data regarding the differences in their frequencies that arise due to ethnicity.<sup>11, 12)</sup>

The gr/gr deletion, a partial deletion in the AZFc region, involves 1.6 megabases.<sup>9)</sup> The frequency of this dele-

tion is associated with ethnic variations, and this deletion has been reported in 10%-15% of cases of male infertility in Africans and Asians and in less than 5% of cases of male infertility in other ethnicities.<sup>13)</sup> The relationship between the gr/gr deletion and infertility also involves geographic and ethnic differences.<sup>14, 15)</sup> In fact, this deletion reportedly exhibits a strong relationship in Caucasians but a weak relationship in East Asians.<sup>16)</sup> In a study involving 772 Japanese patients who were mostly tested at infertility clinics, the frequency of the gr/gr deletion was 33.7% (260 people), whereas the analysis of their semen samples failed to establish a trend, with the results ranging from normal to azoospermia.<sup>17)</sup> These studies show that there are ethnic and geographic differences in gr/gr deletion frequency and in the relationship between the gr/gr deletion and infertility. However, at present, very little information regarding the ethnic variations in gr/gr deletions and their effects on ICSI outcomes in East Asian subjects is available.

Thus, in the present study, we determined the effects of gr/gr deletions on the ICSI outcomes and sperm retrieval rate, compared between subjects with the gr/gr deletion and without AZF deletions via the results of TESE, including microdissection-TESE (MD-TESE). Additionally, we determined the AZF deletion frequency in East Asian subjects at our hospital.

## Methods

### Subjects

Of the patients with azoospermia and severe oligospermia who visited our hospital between July 2014 and August 2018, 204 East Asians with a normal male karyotype who underwent AZF testing were selected as subjects. Severe oligospermia was defined as a sperm count per ejaculate of less than  $5 \times 10^6$ . A retrospective study was performed using data available in medical records (ethics committee approval number: A18024).

### AZF test

For the AZF test, the GENOSEARCH™ AZF Deletion with 21 sequence-tagged site (STS) markers was considered. In this test, sY757, which is an X chromosome STS probe, was used as the control, and 20 Y chromosome STS probes were used, which have been designed in accordance with the EAA/EMQN guidelines.<sup>8)</sup> The chromosome regions to which the probes corresponded were as follows: (i) sY1324, sY1316, and sY1714: AZFa regions; (ii) sY1024, sY1967, sY1309, sY3199, sY1233, sY3010, sY2990,

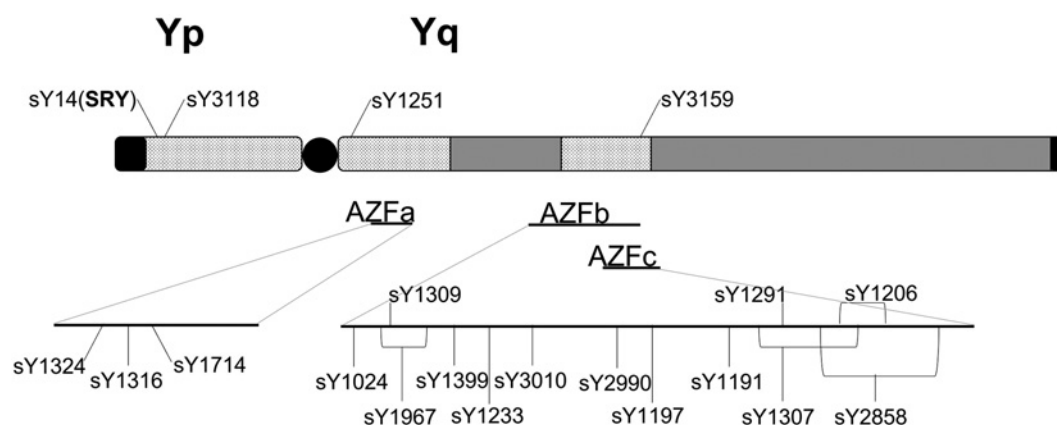


Fig. 1 Distribution of STS markers, primarily in the azoospermia factor (AZF) region of the Y chromosome

As observed in the case of other chromosomes, the centromere on the Y chromosome was located between the long and short arms. The AZF region was located on Yq11. In the AZF tests used in the present study, there were 3 sequence-tagged site (STS) markers corresponding to the AZFa region and 13 STS markers corresponding to the AZFb and AZFc regions. Furthermore, diagnoses were formulated based on the results with the indicated markers using the data provided in the judgment table.

sY1197, sY1191, sY1291, sY1307, sY1206, and sY2858: AZFb and AZFc regions; (iii) sY14 and sY3118: short arm of the Y chromosome, with sY14 corresponding to the *SRY* gene; and (iv) sY1251 and sY3159: long arm of the Y chromosome, except for the AZF region, with sY1251 corresponding to a region close to the Y chromosome centromere and sY3159 corresponding to the euchromatin terminus (Fig. 1).<sup>18)</sup> Using the judgment table available for these probes (Table 1), the AZF test results were determined.

### ICSI

For most subjects for whom sperm retrieval by TESE (including MD-TESE) was feasible, the sperm samples were frozen. In the subjects for whom sperm retrieval was performed, controlled ovarian stimulation was performed with their sexual partners, which was followed by oocyte retrieval and ICSI. Concerning the assessment of fertilization, a fertilized oocyte has been observed to have two pronuclei on Day 1 after ICSI. The embryo develops into a blastocyst on Day 5 or Day 6. The fertilization rate was calculated as the percentage of injected oocytes that transformed into two pronuclei. The blastocyst formation rate was set at Day 5 or Day 6 with a blastocyst (which refers to the embryonic stage consisting of an endocytic mass that becomes the fetus and an ectoderm cell that becomes the placenta) divided by the number of two pronuclei embryos. The decision as to whether blastocysts and early

embryos obtained by ICSI should be transferred while fresh or frozen was made based on the ovarian stimulation method and the endometrial condition.

### Statistical analysis

Data were expressed as the median (range). All data were subjected to analysis using Stata™, version 16 (Stata-Corp LLC, College Station, TX, USA). We used the Mann-Whitney U test for continuous data because all data followed a non-normal distribution and the Fisher's exact test for categorical data. Differences with *p*-values of <0.05 were considered statistically significant (\**p* < 0.05; \*\**p* < 0.01).

## Results

### Patients' characteristics

The AZF test was performed for 253 patients with azoospermia or severe oligospermia. Therefore, excluding the 44 karyotypically abnormal patients and the 5 patients who were not East Asians, the final number of East Asians subjects was 204. Of these 204 subjects, 196 presented with azoospermia, and 200 of these 204 subjects were Japanese. Of the 204 patients, 83 (40.7%) exhibited AZF deletions, 118 had no deletions, and 3 presented with an undetermined result (Fig. 2). The categorization of the AZF deletions is shown in Table 2. The following cases were found: (i) P5/distal P1 deletion with complete AZFb + c deletion, (ii) P5/proximal P1 deletion with complete AZFb-

Table 1 AZF test results obtained using the judgment table based on STS markers

X chromo- some	Y chromosome														eu- chro- matin			
	Yp	Cen- tro- mere		AZFa		AZFb + AZFc												
	sY757	sY14	sY3118 sY1251 sY1324 sY1316 sY1714 sY1024 sY1967 sY1309 sY3199 sY1233 sY3010 sY2990 sY1197 sY1191 sY1291 sY1307 sY1206 sY2858 sY3159															
Deletion ( - )	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AZFa deletion	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AZFb deletion (P5/Proximal P1)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AZFb + c deletion (P5/distal P1)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AZFb + c deletion (P5/distal P1)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AZFc deletion (b2/b4)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AZFc deletion (b2/b4)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-1 (P5 + P4)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-2 (P5 + P4)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-3 (partial AZFb)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-4 (partial AZFb)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-5 (P3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-6 (P3 + P2 + P1)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-7 (P3 + P2 + P1)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-8 (b1/b3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-9 (P3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-10 (P3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-11 (b2/b3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-12 (gr/gr)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ym-13 (P1)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

\*: Not involved in the judgment

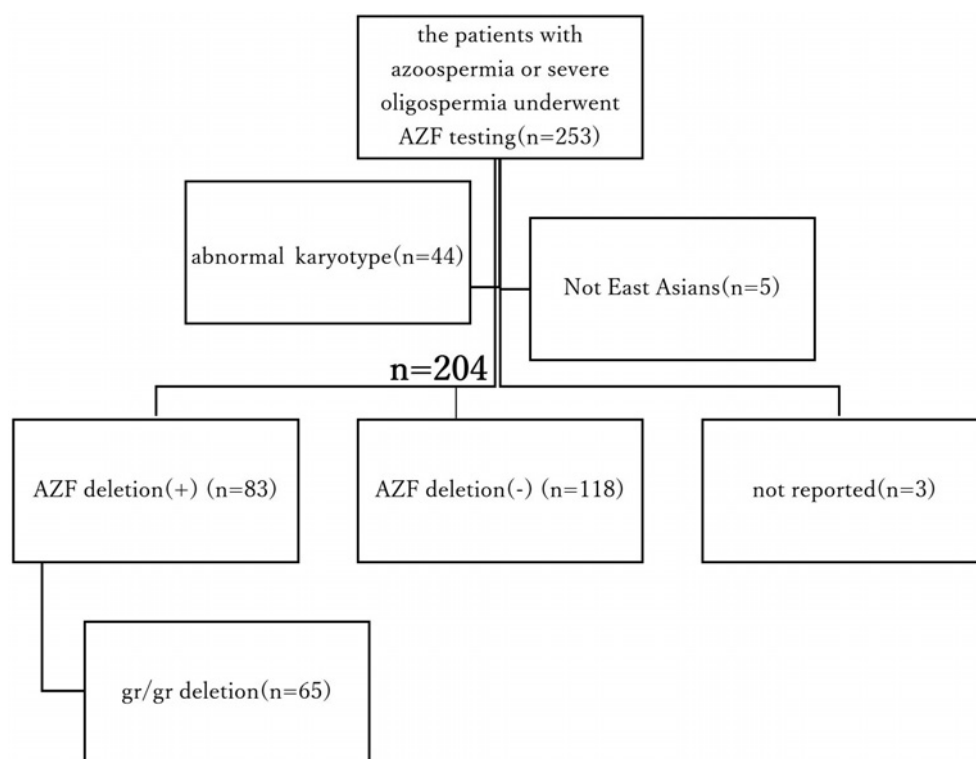


Fig. 2 Categorization of subjects

AZF tests were performed for 253 patients with azoospermia or severe oligospermia. The number of subjects, excluding the 44 with karyotypes that were not 46,XY and/or were outside the normal range of variation and the 5 that were not East Asians, was 204. Of these 204 subjects, 83 exhibited AZF deletions (including 65 with gr/gr deletions), 118 did not harbor deletions, and 3 presented with an undetermined status.

Table 2 Categorization of AZF deletions

AZF deletions	n (%)
AZFb + c deletion (P5/distal P1)	3 (3.6%)
AZFb deletion (P5/proximal P1)	3 (3.6%)
AZFc deletion (b2/b4)	6 (7.2%)
AZFb + c deletion (Ym-9 P3)	2 (2.4%)
AZFc deletion (Ym-12 gr/gr)	65 (78.3%)
AZFc deletion (Ym-11 b2/b3)	3 (3.6%)
AZFc deletion (Ym-8 b1/b3)	1 (1.2%)
Total	83 (100%)

deletion, and (iii) b2/b4 deletion with complete AZFc deletion. Additionally, there were cases of partial AZFc deletion, such as b1/b3, b2/b3, and gr/gr deletions, in which only a relatively small region of the AZF was deleted. Partial AZFc deletions demonstrated the highest frequency, and gr/gr deletions were specifically found in 65 subjects (78.3% of the subjects with AZF deletions).

The backgrounds of the patients in the gr/gr deletion and no-AZF-deletion groups are presented in Table 3.

There were no significant differences between the gr/gr deletion and no-AZF-deletion groups in terms of age, testicular volume, serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, and testosterone levels.

#### Sperm retrieval rates

Further, TESE, including MD-TESE, was performed for a total of 37 patients in the gr/gr deletion group (56.9%) and 77 patients in the no-AZF-deletion group (65.3%). Sperm samples were successfully retrieved from 18 patients (48.6%) in the gr/gr deletion group and from 36 patients (46.8%) in the no-AZF-deletion group (Table 4). No significant difference was found between the no-AZF-deletion and gr/gr deletion groups regarding the percentage of cases requiring TESE and the sperm retrieval rate.

#### Partners' characteristics

In couples for whom sperm retrieval was successful, oocyte retrieval was performed at the authors' hospital after TESE was performed for 34 cycles in 15 subjects from the gr/gr deletion group and for 62 cycles in 29 subjects from

Table 3 Clinical characteristics of patients

	gr/gr deletion ( + ) (n = 65)	AZF deletion ( - ) (n = 118)	p-value
Age	37.2 (25-59)	35.5 (19-61)	.15
Testicular volume (mL)	10.5 (4-20)	11.3 (2-25)	.47
FSH (mIU/mL)	18.7 (2.7-53.0)	19.2 (2.1-94.1)	.75
LH (mIU/mL)	8.37 (1.2-24.4)	8.66 (2.1-43.4)	.78
Testosterone (ng/mL)	4.53 (0.95-9.88)	4.15 (0.27-9.34)	.22

FSH: follicle-stimulating hormone

LH: luteinizing hormone

Data are expressed as the median (range).

Differences between the groups were evaluated by the Mann-Whitney U test.

Table 4 Comparison of sperm retrieval rates

	gr/gr deletion ( + ) (n = 65)	AZF deletion ( - ) (n = 118)	p-value
TESE <sup>a</sup> required	37 (56.9%)	77 (65.3%)	.34
MD-TESE	29	66	
simple TESE	8	11	
sperm retrieved	18 (18/37 = 48.6%)	36 (36/77 = 46.8%)	1
MD-TESE	12 (12/29 = 41.4%)	26 (26/66 = 39.4%)	
simple TESE	6 (6/8 = 75.0%)	10 (10/11 = 90.9%)	

TESE: testicular sperm extraction

MD-TESE: microdissection-testicular sperm extraction

Data are expressed as the number (%).

Differences between the groups were evaluated by the Fisher's exact test.

<sup>a</sup>TESE includes MD-TESE and simple TESE.

Table 5 Comparison of the background factors and oocyte retrieval cycles of the subjects' sexual partners

	gr/gr deletion ( + ) (n = 15)	AZF deletion ( - ) (n = 29)	p-value
Maternal age	34.3 (25-43)	31.7 (24-38)	.04 * †
AMH (ng/mL)	4.81 (0-12.2)	4.76 (0.98-15.2)	.97 †
Oocyte retrieval cycles	34	62	
fresh TESE	1	5	
freeze TESE	33	57	
Use of Pentoxifylline	31/31 (100%)	58/60 (96.7%)	.55 ‡
Use of Calcium ionophore	23/31 (74.2%)	40/60 (66.7%)	.63 ‡

AMH: anti-Müllerian hormone

TESE: testicular sperm extraction

Data are expressed as the median (range) or the number (%).

Differences between the groups were evaluated by the Mann-Whitney U test † or the Fisher's exact test ‡ (\* $p < 0.05$ ).

the no-AZF-deletion group. Next, ICSI was performed using the sperm retrieved via TESE (Table 5). There were no significant differences between the two groups in

terms of the anti-Müllerian hormone (AMH) levels in the sexual partners of the subjects. The partners of the subjects in the gr/gr deletion groups were significantly older

Table 6 Comparison of intracytoplasmic sperm injection (ICSI) outcomes

	gr/gr deletion ( + ) (n = 15)	AZF deletion ( - ) (n = 29)	p-value
Oocyte retrieval cycles	34	62	
Fertilization rate	85/155 (54.8%)	326/490 (66.5%)	<0.01 **
Blastocysts/2PN	23/85 (27.1%)	80/326 (24.5%)	.74
Number of cases of fresh ETs or embryo freezing	12/15 (80%)	27/29 (93.1%)	.32
Biochemical pregnancies/ETs	13/24 (54.2%)	35/89 (39.3%)	.25
Clinical pregnancies/ETs	11/24 (45.8%)	28/89 (31.5%)	.28
Live births/ETs	11/24 (45.8%)	20/89 (22.4%)	.04 *

PN: pronucleus

ET: embryo transfer

Data are expressed as the number (%).

Differences between the groups were evaluated by the Fisher's exact test (\* $p < 0.05$ ; \*\* $p < 0.01$ ).

than those of the subjects in the no-AZF-deletion group.

### ICSI outcomes

The outcomes of ICSI are presented in Table 6. The fertilization rate of the subjects who underwent TESE was 54.8% (85/155) for the gr/gr deletion group and 66.5% (326/490) for the no-AZF-deletion group. The sperm samples from the subjects in the gr/gr deletion group showed a significantly lower fertilization rate than those from the subjects in the no-AZF-deletion group ( $p < 0.01$ ). There were no significant differences in the blastocyst formation rate per embryo or clinical pregnancy rate per embryo transfer cycle. After the clinical achievement of pregnancy, spontaneous abortion was not experienced by the partners of any of the subjects in the gr/gr deletion group. Furthermore, the live birth rate per transfer was significantly elevated in the gr/gr deletion group.

### Discussion

The fertilization rate in East Asian subjects with normal karyotype who underwent TESE (including MD-TESE) was significantly lower for the gr/gr deletion group than for the no-AZF-deletion group. However, there were no significant differences between the two groups in terms of the blastocyst formation and clinical pregnancy rates.

First, the fertilization rate via TESE (including MD-TESE) was significantly lower for the gr/gr deletion group than for the no-AZF-deletion group. In a reported meta-analysis of the effects of the Y chromosome microdeletions on ejaculated sperm and sperm obtained via TESE, the fertilization rate of the Y chromosome microdeletions was found to be significantly reduced (odds ratio: 0.75; 95% con-

fidence interval: 0.63-0.88;  $p < 0.01$ ).<sup>19)</sup> This report had no limitations in terms of race and included not only testicular sperm but also ejected sperm. Because the entire Y chromosome microdeletion is targeted, deletions outside the AZF regions and AZF deletions other than gr/gr deletions were also included, but this study suggests that the gr/gr deletion does affect fertility. Furthermore, in relation to AZFc microdeletions, the fertilization rate with testicular sperm was significantly lower than that observed with ejaculated sperm (43.7% vs. 66.4%). Additionally, the fertilization rate in the subjects who were subjected to the use of testicular sperm was found to be significantly lower in case of the AZFc microdeletion group than in case of the no-AZF-deletion group (43.7% vs. 53.6%).<sup>20)</sup> This previous report targeted AZFc microdeletions and did not focus on gr/gr deletions, but it suggests that gr/gr deletions may influence the fertilization rate to some extent.

Furthermore, in this study, maternal age may be another factor that reduced the fertilization rate. The age of the partners of the subjects in the gr/gr deletion group was significantly higher than that of the partners of the subjects in the no-AZF-deletion group. However, the median age difference between the partners of the subjects from the two groups is 2.6 years. As such, we believe that the two-year period in the early 30s did not have a notable effect on egg quality. In addition, because there was no significant difference in the AMH levels between the two groups, we do not believe that the statistical significance of the maternal age between the two groups was significantly involved in the decrease in fertility.

Second, it was revealed that there was no difference in



the blastocyst formation rate and clinical pregnancy rate after fertilization between the two groups in case of the subjects from the gr/gr deletion group. A meta-analysis of Y chromosome microdeletion reported no significant differences in good embryo rate, clinical pregnancy rate, early miscarriage rate, miscarriage rate, or live birth rate.<sup>19)</sup> In addition, it has been reported that there is no significant difference between the clinical pregnancy rate and miscarriage rate in subjects with AZFc microdeletion.<sup>20)</sup> These reports show that once the egg is fertilized, there is no difference in the ICSI or pregnancy results. This is consistent with the fact that there was no difference in the blastocyst formation rate and clinical pregnancy rate in the present study. In the present study, miscarriage did not occur in the gr/gr deletion group, and the live birth rate per transfer in this group was significantly higher than that in the no-AZF-deletion group. The factor most related to the miscarriage rate is the age of the partner, and according to the JSOG ART report, the miscarriage rate in the group of patients in their 30s is estimated to be 17%-20%.<sup>21)</sup> In this previous study, the gr/gr deletion group showed a significantly higher maternal age and a higher miscarriage rate. However, the miscarriage rate was lower and the live birth rate per transfer was higher in the gr/gr deletion group. It is unlikely that the gr/gr deletion will affect miscarriage, and it is highly possible that other factors are involved, but the examination of these aspects is outside the scope of this study.

Third, there were no significant differences in the sperm retrieval rates achieved via TESE (including MD-TESE) between the gr/gr deletion and no-AZF-deletion groups. In a previous study, no significant differences were reported between the sperm retrieval rates in the groups with and without the gr/gr deletion.<sup>22)</sup> A similar result was observed in the present study. Conversely, it has been reported that the sperm retrieval rate was 60.0%-74.5% for the entire-AZFc deletion group (including the subjects with the gr/gr deletion), which was significantly higher than that for the group without the deletion.<sup>20, 22, 23)</sup> The mechanism whereby the sperm retrieval rate increases is unknown. However, there are no reports showing that sperm recovery is lower in the gr/gr deletion group; this information may be important during genetic counseling for individuals with the gr/gr deletion.

Fourth, among the 204 karyotypically normal subjects with oligospermia or azospermia, 40.7% (n = 83) exhibited AZF deletions, of whom 78.3% (n = 65) had gr/gr deletions.

In Japan, 11.8% of male patients with infertility present with AZF deletions, and the gr/gr deletion frequency has been reported to be markedly affected by ethnicity.<sup>13)</sup> The present study was performed on East Asians, which constitute an ethnic group with a high frequency of AZF deletion. However, the AZF deletion frequency among patients with azospermia and oligospermia at the authors' center herein was markedly higher than that reported in previous studies. Among the AZF deletions documented, the frequencies were as follows: AZFc deletion, 80%; AZFa deletion, 0.5%-4%; AZFb deletion, 1%-5%; and AZFb + c deletion, 1%-3%.<sup>7)</sup> Approximately the same frequencies were observed in the present study. It has been reported that the gr/gr deletion is highly common in East Asian subjects. The frequency of the gr/gr deletion in Japanese people has been reported to be 33.7% in infertility clinics.<sup>17)</sup> In the present study, the frequency of the gr/gr deletion was found to be approximately the same (31.9%).

The present study has a few limitations. It was performed at a single institution, and oocyte retrieval was performed for 44 subjects at the authors' hospital after the successful retrieval of sperm. Hence, the possibility of selection bias could not be ruled out. However, the performance of the study at a single institution facilitated treatment-related standardization, and errors associated with the treatment of each subject may not have occurred.

In conclusion, to the best of our knowledge, the fertilization rates of TESE-derived sperms were significantly lower for the East Asian subjects with the gr/gr deletion. TESE-derived sperms from men with the gr/gr deletion were morphologically completely mature spermatozoa with some functional or quality issues. The AZF gr/gr deletion may affect the fertilizing capacity of testicular sperms in East Asians. The sperm retrieval rate of these subjects was similar to that of the no-AZF-deletion group (despite the presence of the gr/gr deletion). Once the fertilized egg was formed, the subsequent ICSI and pregnancy results for the subjects with the gr/gr deletion were similar to those for the individuals without AZF deletions. Our findings will be useful in providing explanations prior to ART for subjects with the gr/gr deletion.

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**Authors' contribution:** YT and YK designed the study. YT, YK, YF, HK, and KN were involved in the data collection of this study. YT, YK, and MN wrote the manuscript. All authors read and approved the final manuscript.

**Ethics statement:** Human rights statements and informed consent: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent for the AZF test was obtained from all participants included. The participants were given the opportunity to decline using their test results for the present study.

**Animal rights:** This article does not contain any studies with animal subjects performed by the any of the authors.

This report was approved by the Ethics Committee of the Faculty of Medicine, Toho University (No. A18024).

**Conflicts of interest:** None declared.

## References

- 1) Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. *Int J Androl*. 2004; 27: 240-9.
- 2) Krausz C, Rajpert-De Meyts E, Frydelund-Larsen L, Quintana-Murci L, McElreavey K, Skakkebaek NE. Double-blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: microdeletions are specific for spermatogenic failure. *J Clin Endocrinol Metab*. 2001; 86: 2638-42.
- 3) Simoni M, Tüttelmann F, Gromoll J, Nieschlag E. Clinical consequences of microdeletions of the Y chromosome: the extended Münster experience. *Reprod Biomed Online*. 2008; 16: 289-303.
- 4) Lo Giacco D, Chianese C, Sánchez-Curbelo J, Bassas L, Ruiz P, Rajmil O, et al. Clinical relevance of Y-linked CNV screening in male infertility: new insights based on the 8-year experience of a diagnostic genetic laboratory. *Eur J Hum Genet*. 2014; 22: 754-61.
- 5) Achermann APP, Pereira TA, Esteves SC. Microdissection testicular sperm extraction (micro-TESE) in men with infertility due to nonobstructive azoospermia: summary of current literature. *Int Urol Nephrol*. 2021; 53: 2193-210.
- 6) Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum Genet*. 1976; 34: 119-24.
- 7) Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kisesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet*. 1996; 5: 933-43.
- 8) Krausz C, Hoefsloot L, Simoni M, Tüttelmann F, Academy European, of Andrology, European Molecular Genetics Quality Network. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. *Andrology*. 2014; 2: 5-19.
- 9) Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T, et al. Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. *Nat Genet*. 2003; 35: 247-51.
- 10) Krausz C, Casamonti E. Spermatogenic failure and the Y chromosome. *Hum Genet*. 2017; 136: 637-55.
- 11) Sen S, Pasi AR, Dada R, Shamsi MB, Modi D. Y chromosome microdeletions in infertile men: prevalence, phenotypes and screening markers for the Indian population. *J Assist Reprod Genet*. 2013; 30: 413-22.
- 12) Yousefi-Razin E, Nasiri MJ, Omrani MD. Frequency of Y chromosome microdeletions among Iranian infertile men with azoospermia and severe oligozoospermia: a meta-analysis. *J Reprod Infertil*. 2016; 17: 208-12.
- 13) Colaco S, Modi D. Genetics of the human Y chromosome and its association with male infertility. *Reprod Biol. Endocrinol*. 2018; 16: 14.
- 14) Ferlin A, Tessari A, Ganz F, Marchina E, Barlati S, Garolla A, et al. Association of partial AZFc region deletions with spermatogenic impairment and male infertility. *J Med Genet*. 2005; 42: 209-13.
- 15) Giachini C, Laface I, Guarducci E, Balercia G, Forti G, Krausz C. Partial AZFc deletions and duplications: clinical correlates in the Italian population. *Hum Genet*. 2008; 124: 399-410.
- 16) Stouffs K, Lissens W, Tournay H, Haentjens P. What about gr/gr deletions and male infertility? Systematic review and meta-analysis. *Hum Reprod Update*. 2011; 17: 197-209.
- 17) Sin H-S, Koh E, Shigehara K, Sugimoto K, Maeda Y, Yoshida A, et al. Features of constitutive gr/gr deletion in a Japanese population. *Hum Reprod*. 2010; 25: 396-403.
- 18) Iijima M, Koh E, Izumi K, Taya M, Maeda Y, Kyono K, et al. New molecular diagnostic kit to assess Y-chromosome deletions in the Japanese population. *Int J Urol*. 2014; 21: 910-6.
- 19) Li X, Li X, Sun Y, Han J, Ma H, Sun Y. Effect of Y Chromosome microdeletions on the pregnancy outcome of assisted reproduction technology: a meta-analysis. *Reprod Sci*. 2021; 28: 2413-21.
- 20) Yamaguchi K, Ishikawa T, Mizuta S, Takeuchi T, Matsubayashi H, Kokeguchi S, et al. Clinical outcomes of microdissection testicular sperm extraction and intracytoplasmic sperm injection in Japanese men with Y chromosome microdeletions. *Reprod Med Biol*. 2020; 19: 158-63.
- 21) Ishihara O, Jwa SC, Kuwahara A, Katagiri Y, Kuwabara Y, Hamatani T, et al. Assisted reproductive technology in Japan: a summary report for 2018 by the Ethics Committee of the Japan Society of Obstetrics and Gynecology. *Reprod Med. Biol*. 2021; 20: 3-12.
- 22) Iijima M, Shigehara K, Igarashi H, Kyono K, Suzuki Y, Tsuji Y, et al. Y chromosome microdeletion screening using a new molecular diagnostic method in 1030 Japanese males with infertility. *Asian J Androl*. 2020; 22: 368-71.
- 23) Stahl PJ, Masson P, Mielnik A, Marean MB, Schlegel PN, Paduch DA, et al. A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. *Fertil Steril*. 2010; 94: 1753-6.

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