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Infertility treatment for patients having a microdeletion of azoospermic factor (AZF)

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ABSTRACT

In genetic causes of male infertility, Y chromosome microdeletions are the second most common after Klinefelter's syndrome. Although sperm recovery rate is relatively high for subjects with azoospermic factor (AZF) c chromosome microdeletion, intracytoplasmic sperm injection (ICSI) results using retrieved sperm has been reported to be poor. We retrospectively examined the infertility treatment for subjects with AZF microdeletion. From October 2017 to September 2020, chromosomal examination of 67 azoospermic subjects and 12 cryptozoospermia were performed. Of these, twenty-three subjects (29.1%) had AZF microdeletion. Twelve subjects with AZFc microdeletion and one subtype with unknown classification (Ym-9; P3 deletion) received sperm retrieval surgery due to azoospermia. Two subjects obtained motile sperm by microscopic epididymal sperm aspiration (MESA) and four subjects by microscopic testicular sperm extraction (micro-TESE). Pregnancy and healthy delivery were achieved in 6 of 14 subject (42.9%; including one twin) using ICSI. This was comparable with previous reports. Since there were two cases of obstructive azoospermia, we employed MESA to avoid testicular damage. Following observation of the testis and epididymis under operative microscope, a decision was made to perform sperm retrieval surgery to avoid unnecessary testicular damage. Furthermore, since AZFc microdeletion is passed to the next generation, long term follow-up is necessary.

Keywords: AZF microdeletion, azoospermia, cryptozoospermia, MESA, micro-TESE

Abbreviations:
AZF: azoospermic factor
ICSI: intracytoplasmic sperm injection
MESA: microscopic epididymal sperm aspiration
micro-TESE: microscopic testicular sperm extraction
NOA: non-obstructive azoospermia
OA: obstructive azoospermia

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INTRODUCTION

Male factor infertility accounts for almost half of the cases in a couple desiring a child. These male factors problems can be ascribed to infection, immunological factors, anatomical malformations, or environmental insult. Besides these factors, genetic abnormalities can also cause male infertility. In conjunction with the use of intracytoplasmic sperm injection (ICSI) and sperm retrieval technique, patients with chromosomal abnormality are now able to conceive.[1,2] In addition, recent studies have shown that spermatozoa can be retrieved from men with azoospermic factor (AZF) c microdeletion, thus increasing the chances of conception.[3,4]

Since patients with AZF microdeletion are generally considered as having non-obstructive azoospermia (NOA), microscopic testicular sperm extraction (micro-TESE) is employed for sperm extraction surgery. In the present study, we retrospectively evaluated the clinical characteristics, sperm retrieval rates, and birth rates for patients with Y chromosome microdeletions in our institute.

PATIENTS AND METHODS

From October 2017 to September 2020, we performed chromosomal examination for 67 azoospermic subjects and 12 cryptozoospermia (defined as containing less than 1 million sperms per mL of ejaculate and occasionally zero sperm count) in Asada Ladies Clinic. Patients' characteristics are shown in Table 1. Physical examination revealed bilateral absent vas deferens in one subject. Clinically prominent varicocele was not observed. One azoospermic subject had history of chronic myeloid leukemia (CML) treated with chemotherapy and bone marrow transplantation from his sibling at nine-years-old. Sperm retrieval surgery underwent to 13 azoospermic subjects and one cryptozoospermic subject who could not cryopreserve sperm after repeated ejaculation. ICSI was attempted by using surgically retrieved or ejaculated, and evaluated the result of pregnancy and delivery.

Y chromosome microdeletions obtained from peripheral blood lymphocytes were tested at a clinical laboratory (Integrated GENETICS). The analysis system is comprised of the primer which is specific for 20 kinds of sequence tagged sites (STS). These primers amplify a DNA

Table 1 Patients' characteristics

	n=23
Age (year)	35 (25–54)
Spouse age (year)	32 (25–43)
Testicular volume (right/ left) (mL)	8 (2–20) / 7 (2–18)
LH (mIU/mL)	6.5 (2.3–27.1)
FSH (mIU/mL)	16.0 (3.7–51.7)
Testosterone (ng/mL)	2.80 (1.16–6.70)
Zinc (μg/dL)	84 (62–131)
BMI (Kg/m^2)	23.1 (19.3–27.8)

Each data was presented as median.
LH: luteinizing hormone
FSH: follicle stimulating hormone
BMI: body mass index

fragment by the polymerase chain reaction (PCR) method from a Y chromosome-specific domain. The presence or absence of each Y specific sequence and the presence of an internal control is determined by gel electrophoresis and ethidium bromide staining. This test was developed and its performance characteristics determined by Esoterix Genetic Laboratories, LLC.

RESULTS

Amongst 67 azoospermic and 12 cryptozoospermic subjects, 23 (29.1%) subjects showed AZF microdeletion. Type of AZF microdeletions were AZFa in 2, AZFb (1), AZFb+c (1), AZFc (18), and unknown classification as AZF Ym-9 (P3 deletion) microdeletion (1), respectively. Correlation among semen analysis, chromosomal G band, and type of AZF microdeletion are shown in Table 2. Regarding to subtype of AZFc microdeletion, Ym-12 gr/gr microdeletion was most common in 12 subjects, and Ym-11 b2/b4 microdeletion (1), unknown (3), respectively.

Table 2 Semen analysis, chromosomal G band and type of AZF microdeletion

Semen analysis (n)	G band	Type of AZF microdeletion (n)
Azoospermia (19)	46XY/46XY inv(9)(p11q13)	AZFa (2)
	46XY	AZFb (P5/proximal P1) (1)
	46,X,der(Y) (Ypter→Yq11.23::Yp11.2→Ypter)	AZFb+c (1)
	46XY	AZF Ym-9 (P3) (1)
	46XY/ 46XY inv(9)(p12:q13)	AZFc unknown subtype (2)
	46XY	AZFc Ym-12 gr/gr (12)
Cryptozoospermia (4)	46XY	AZFc Ym-12 gr/gr (2)
	46XY	AZFc Ym-11 b2/b4 (1)
	46XY	AZFc unknown subtype (1)

AZF: azoospermic factor

Sperm retrieval surgery

A total of 14 subjects, 13 with azoospermia and one cryptozoospermia underwent sperm retrieval surgery. Type of AZFc microdeletions for azoospermia were Ym 12 gr/gr in ten, unknown in two, and AZF Ym-9 (P3 deletion) in one. One cryptozoospermic subject showed a Ym 12 gr/gr microdeletion. Of the 13 azoospermic subjects, 11 underwent micro-TESE and motile sperm were recovered in 4 cases (36.4%) including one with a history of CML in infancy. Since two azoospermic subjects showed dilated epididymal tubule under an operating microscope, we employed microscopic epididymal sperm aspiration (MESA), and both cases recovered motile sperm. Of these, the subject with AZF Ym-9 (P3 deletion) preoperatively was diagnosed as having congenital absence of vas deferens by careful palpation, and another had who showed AZFc Ym-12 gr/gr microdeletion had tentatively diagnosed an epididymal obstruction because of normal testicular volume and gonadotropins. On the other hand, one cryptozoospermia who failed cryopreservation of ejaculated sperm after repeated trials received micro-TESE and recovered motile sperm.

ICSI results

One patient with cryptozoospermia of unknown AZFc subtype had a healthy girl by ICSI using cryopreserved ejaculated sperm. On the other hand, four pregnancies and subsequent deliveries were obtained by TESE-ICSI. Of these, a cryptozoospermic subject who underwent TESE had twin healthy girls. Two of the MESA-ICSI subjects had the birth of a healthy boy and girl. Thus, pregnancy and delivery were achieved in 6 of the 14 subjects (42.9%) who underwent surgical sperm retrieval. Two boys and five girls (including one twin) were born. Details of AZF subtypes, sperm retrieval surgery, and ICSI results are shown in Table 3.

Table 3 Sperm retrieval surgery and result on each case

	Type of AZF microdeletion	Surgery	Sperm recovery	Pregnancy/delivery
Case-1	AZF Ym-9 (P3)	MESA	Yes	Yes/healthy girl
Case-2	AZFc Ym-12 gr/gr	m-TESE	No	–
Case-3	AZFc Ym-12 gr/gr	m-TESE	No	–
Case-4	AZFc unknown subtype	m-TESE	Yes	Yes/healthy boy
Case-5	AZFc Ym-12 gr/gr	m-TESE	No	–
Case-6	AZFc Ym-12 gr/gr	m-TESE	No	–
Case-7	AZFc Ym-12 gr/gr	m-TESE	Yes	No
Case-8	AZFc Ym-12 gr/gr	m-TESE	Yes	Yes/healthy girl
Case-9	AZFc Ym-12 gr/gr	MESA	Yes	Yes/healthy boy
Case-10	AZFc unknown subtype	m-TESE	No	–
Case-11	AZFc Ym-12 gr/gr	m-TESE	No	–
Case-12	AZFc Ym-12 gr/gr	m-TESE	No	–
Case-13	AZFc Ym-12 gr/gr	m-TESE	Yes	Yes/healthy girl
*Case-14	AZFc Ym-12 gr/gr	m-TESE	Yes	Yes/healthy girl (twin)

*Case-14 was cryptozoospermic subject who failed cryopreservation of ejaculated sperm after repeated trials.
MESA: microscopic epididymal sperm aspiration
m-TESE: microscopic testicular sperm extraction

DISCUSSION

Y chromosome microdeletions are the second most common genetic cause of male infertility after Klinefelter's syndrome. The locus defined as AZF in Yq11 contains the genes necessary for normal spermatogenesis, and microdeletions in this locus have been correlated with male infertility. The detailed analysis of the Y chromosome in men with azoospermia or cryptozoospermia has resulted in the identification of three regions of the long arm of the human Y chromosome, named AZFa, AZFb and AZFc, that are currently deleted in men with otherwise unexplained spermatogenic failure.[5] The importance of careful evaluation of AZF microdeletions in male infertility before assisted reproduction is clear.[6] Distinct histopathology phenotypes were correlated with the site of the microdeletion, varying from Sertoli cell only (SCO) syndrome in patients with AZFa microdeletions, maturation arrest at meiosis (MA) in cases with AZFb microdeletions, to hypospermatogenesis in patients with AZFc microdeletions.[7,8] Thus, only

subjects with AZFc microdeletion a reconsidered as a candidate for surgical sperm retrieval but this should not be performed for subjects with AZFa, AZFb, and AZFb+c microdeletions. We agree with this consideration, and in the present study, we examined sperm retrieval surgery for AZFc microdeletions except one case of unknown classification as AZF Ym-9 (P3 deletion) microdeletion. AZFc microdeletion is to cause of spermatogenesis disorders due to mainly b2/b4 deletion. On the other hand, partial deletion of AZFc is divided into AZFc gr/gr deletion and AZFc b2/b3, and subjects with AZFc gr/gr microdeletion in Japan is present in almost 30% of all men.[9] Thus, there are various patterns of partial deletion of AZFc, and the clinical picture is also different.

According to the practice committee of the American Society of Reproductive Medicine, the prevalence of Y chromosome microdeletion may be as high as 2% in the general population of unselected men.[10] On the other hand, the European academy of andrology defines the prevalence of AZF microdeletion in unselected men to be far lower, around 1 in 4000 (0.025%).[11] A recent meta-analysis of over 10,000 North American men found that while 5% of cryptozoospermic patients had an AZF microdeletion, the prevalence of these same deletions was less than 1% amongst men with normozoospermia.[12] A large scale Japanese study screened 1030 infertile Japanese men for Y chromosome microdeletion, yielding a prevalence of nearly 7% including all type of AZF microdeletion in the population made up almost exclusively of severely oligozoospermic or azoospermic men. Among the 1030 patients, 4 (5.7%), 4 (5.7%), 10 (14.3%), and 52 (74.3%) had AZFa, AZFb, AZFb+c, and AZFc microdeletions, respectively.[13] The ratio of our subjects with AZF microdeletion was high (23/79=29.1%) when compared with previous data, however, the ratio of AZFc microdeletion was similar (18/23=78.3%).

Iijima et al reported that the sperm recovery rate (SRR) of micro-TESE in patients with AZFc deletions was significantly higher than that in those without AZF deletions (60.0% vs 28.7%, P=0.04). Regarding to subtype of AZFc, in Ym-11 gr/gr deletion, SRR was 18.7%, which was lower than that in those without gr/gr deletion, but was not statistically significant.[13] Tsujimura et al reported that among 60 patients with NOA, the spermatozoa retrieval rate for patients with Y chromosome microdeletion was similar to that of patients without Y chromosome microdeletion (33.3% vs 37.0%).[14] Choi et al also reported that the sperm retrieval rate was similar between patients with no microdeletion and Y chromosome microdeletion (25.6% vs 26.6%, p=0.298).[15] Of the 13 subjects who underwent sperm retrieval surgery in our study, 6 (46.2%) subjects achieved motile sperm recovery (MESA 2/2=100%, micro-TESE 4/11=36.4%). Their subtypes were AZFc Ym-12 gr/gr microdeletion in four, Ym-9 (P3 deletion) in one, and unknown in one. Thus, the correlation of the successful motile recovery with the AZFc subtype seems to be unclear.

Yamaguchi et al concluded in a Japanese study analysis that although successful sperm retrieval was high, ICSI results, especially the fertilization rate was low. When compared with the control group, the fertilization rate was significantly lower in the testicular group with AZFc microdeletions (43.7% vs 53.6%, P <0.001).[16] On the other hand, spermatozoa retrieved from men with AZFc deletions when used for ICSI, produce viable embryos and pregnancy at similar rates as spermatozoa of men without AZFc microdeletions.[17] Nickkholgh et al also reported that AZFc microdeleted spermatogonia behave very similar to normal spermatogonia in vitro and show equal levels in expression of spermatogonial and differentiation markers as spermatogonia from non-deleted control men. They concluded that this indicates the treatment of men with AZFc microdeletions by propagating their spermtogonial stem cell in vitro and autotransplanting them back to the testis is potentially feasible treatment option to restore their fertility.[18] In our study, pregnancy and healthy delivery were achieved in 6 of 14 subject (42.9%; including one twin) using motile sperm obtained by ejaculated, MESA, and micro-TESE. This was comparable with mentioned above previous reports.

Although sperm retrieval rate for the subjects with AZFc microdeletion was similar upon researcher, pregnancy rate was much different.[3,4,14-17] Thus, ICSI results for subjects with AZFc microdeletion are still controversial and accumulation of future cases is necessary.

Azoospermia is classified as either "obstructive azoospermia (OA)" or "NOA," mainly according to the FSH value, testicular volume, chromosomal evaluation, and past history. Since subjects with AZFc microdeletion are considered as NOA, micro-TESE has been employed for sperm retrieval surgery. However, in our case, two subjects who underwent MESA showed one was epididymal obstruction, and another was absent of vas deferens. Their type of AZF microdeletion was AZFc Ym-12 gr/gr, and AZF Ym-9 (P3 deletion). Thus, it should be considered that azoospermia has a different pathology from AZF microdeletion. Although MESA requires microsurgical skill, aspirated MESA specimen does not have any special requirements, such as mincing the tissue. It leads to a reduction in the amount of laboratory work that is needed for cryopreservation. Furthermore, MESA results in better fertilization and pregnancy rates than TESE.[19] van Wely et al also reported that in the first ICSI cycles of couples with obstructive azoospermia, the use of epididymal spermatozoa resulted in a significantly higher live birth rate than did the use of testis spermatozoa.[20] There is concern about the long-term complications including of testicular damage by TESE namely testicular atrophy and decreased testosterone. Testosterone is a lifelong important hormone not only at the time of fertility potential. Since MESA does not require cutting the testis, it does not cause cellular damage that could lead to a decrease in testosterone. We believe that MESA should be employed for OA to avoid unnecessary testicular damage.

The AZF test was considered necessary to avoid unnecessary surgery by finding AZF other than AZFc, and in the case of AZFc, to know before surgery that AZFc will be transmitted to the next generation of boys. The possibility of sperm recovery by the subtype of AZFc remained unclear from this study because the number of cases is small. However, long term follow-up is necessary including an examination of fertility in the next generation.

CONCLUSIONS

Pregnancy and delivery by ART can be expected if sperm are obtained even in the case of AZF microdeletions. Subjects with AZFc microdeletion were usually regarded as NOA, but OA was also apparent in this study. We must be especially careful of the testis and epididymis under operative microscope and then to decide the appropriate sperm retrieval surgery to avoid unnecessary testicular damage. Furthermore, since AZFc microdeletion is inherited to the next generation in males, long term follow-up is necessary.

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DISCLOSURE STATEMENT

Human rights and informed consent statements

All procedures completed were done in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national), and with the Helsinki Declara-

tion of 1964 and its later amendments. Informed consent was obtained from all patients for the purpose of inclusion in this study.

STATEMENT OF ETHICS

The protocol for this research project, including its use of human subjects, was approved by a suitably constituted Ethics Committee. (Our approval number: 2020-17, Date of approval by the Ethical Review Committee: 2020/9/30)

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Each author has no COI with regard to this manuscript.

REFERENCES

1 Kent-First MG, Kol S, Muallem A, et al. The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers. *Mol Hum Reprod.* 1996;2(12):943–950. doi:10.1093/molehr/2.12.943.
2 Oliva R, Margarit E, Ballescá JL, et al. Prevalence of Y chromosome microdeletions in oligospermic and azoospermic candidates for intracytoplasmic sperm injection. *Fertil Steril.* 1998;70(3):506–510. doi:10.1016/s0015-0282(98)00195-2.
3 van Golde RJ, Wetzels AM, de Graaf R, Tuerlings JH, Braat DD, Kremer JA. Decreased fertilization rate and embryo quality after ICSI in oligozoospermic men with microdeletions in the azoospermia factor c region of the Y chromosome. *Hum Reprod*. 2001;16(2):289–292. doi:10.1093/humrep/16.2.289.
4 Oates RD, Silber S, Brown LG, Page DC. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. *Hum Reprod.* 2002;17(11):2813–2824. doi:10.1093/humrep/17.11.2813.
5 Vogt PH, Edelmann A, Kirsch S, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet.* 1996;5(7):933–943. doi:10.1093/hmg/5.7.933.
6 Gonçalves C, Cunha M, Rocha E, et al. Y-chromosome microdeletions in nonobstructive azoospermia and severe oligozoospermia. *Asian J Androl.* 2017;19(3):338–345. doi:10.4103/1008-682X.172827.
7 Stahl PJ, Masson P, Mielnik A, Marean MB, Schlegel PN, Paduch DA. A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. *Fertil Steril.* 2010;94(5):1753–1756. doi:10.1016/j.fertnstert.2009.09.006.
8 Y Chromosome Consortium. A nomenclature system for the tree of human Y-chromosomal binary haplogroups. *Genome Res.* 2002;12(2):339–348. doi:10.1101/gr.217602.
9 Koh E, Iijima M, and Namiki M. Azoospermia related genes and the detection kit for Y chromosome microdeletion [in Japanese]. *J Mamm Ova Res.* 2013;30(4):135–144. doi:10.1274/jmor.30.135.
10 Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. *Fertil Steril.* 2015;103(3):e18–e25. doi:10.1016/j.fertnstert.2014.12.103.
11 Krausz C, Hoefsloot L, Simoni M, Tüttelmann F; European Academy 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(1):5–19. doi:10.1111/j.2047-2927.2013.00173.x.
12 Kohn TP, Kohn JR, Owen RC, Coward RM. The prevalence of Y-chromosome microdeletions in oligozoospermic men: a systematic review and meta-analysis of European and North American studies. *Eur Urol.* 2019;76(5):626–636. doi:10.1016/j.eururo.2019.07.033.
13 Iijima M, Shigehara K, Igarashi H, et al. Y chromosome microdeletion screening using a new molecular diagnostic method in 1030 Japanese males with infertility. *Asian J Androl.* 2020;22(4):368–371. doi:10.4103/aja.aja_97_19.
14 Tsujimura A, Matsumiya K, Takao T, et al. Clinical analysis of patients with azoospermia factor deletions by microdissection testicular sperm extraction. *Int J Androl.* 2004;27(2):76–81. doi:10.1046/j.0105-6263.2003.00450.x.

15 Choi DK, Gong IH, Hwang JH, Oh JJ, Hong JY. Detection of Y chromosome microdeletion is valuable in the treatment of patients with nonobstructive azoospermia and oligoasthenoteratozoospermia: Sperm retrieval rate and birth rate. *Korean J Urol.* 2013;54(2):111–116. doi:10.4111/kju.2013.54.2.111.
16 Yamaguchi K, Ishikawa T, Mizuta S, et al. Clinical outcomes of microdissection testicular sperm etraction and intracytoplasmic sperm injection in Japanese men with Y chromosomal microdeletions. *Reprod Med Biol.* 2020;19(2):158–163. doi:10.1002/rmb2.12317.
17 Liu XH, Qiao J, Li R, Yan LY, Chen LX. Y chromosome AZFc microdeletion may not affect the outcomes of ICSI for infertile males with fresh ejaculated sperm. *J Assist Reprod Genet.* 2013;30(6):813–819. doi:10.1007/s10815-013-0009-y.
18 Nickkholgh B, Korver CM, van Daalen SK, van Pelt AM, Repping S. AZFc deletions do not affect the function of human spermatogonia in vitro. *Mol Hum Reprod.* 2015;21(7):553–562. doi:10.1093/molehr/gav022.
19 Hibi H, Sumitomo M, Fukunaga N, Sonohara M, Asada Y. Superior clinical pregnancy rates after microsurgery epididymal sperm aspiration. *Reproduct Med Biol.* 2018;17(1):59–63. doi:10.1002/rmb2.12069.
20 van Wely M, Barbey N, Meissner A, Repping S, Silber SJ. Live birth rates after MESA or TESE in men with obstructive azoospermia: is there a difference? *Hum Reprod.* 2015;30(4):761–766. doi:10.1093/humrep/dev032.