

Case Report

Non-obstructive Azoospermia in Male with Y-Chromosome Microdeletion: A Case Report

Jesselyn Angellee^{1,2,3*} , Agustinus Agustinus^{1,3,4}, Pety Narulita^{1,3}, Markus Christian Hartanto^{1,3}, William William⁵, Sally Suharyani^{1,3} 

¹ Andrology Study Program, Department of Biomedical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

² Halim Fertility Center, Stella Maris Women's and Children's Hospital, Medan, Indonesia

³ Andrology Outpatient Clinic, Dr. Soetomo General Hospital, Surabaya, Indonesia

⁴ Ferina Hospital-Center of Reproductive Medicine, Surabaya, Indonesia

⁵ Department of Medical Biology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia



ARTICLE INFO

Received: October 16, 2024
Accepted: November 19, 2024
Published: December 05, 2024

*) Corresponding author:

E-mail:

jesselyn.angellee2022@fk.unair.ac.id

Keywords:

Male Infertility
Non-Obstructive Azoospermia
Y-Chromosome Microdeletion
Azfb Microdeletion
Reproductive Rights

This is an open access article under the CC BY-SA license
(<https://creativecommons.org/licenses/by-sa/4.0/>)



Abstract

Male factors have contributed to at least 50% of all infertility cases worldwide. Numerous factors causing male infertility have been identified, one of which is azoospermia due to genetic defects. The detection of Y-chromosome microdeletion may assist in diagnosing male infertility as well as predicting the success rate of testicular sperm extraction. A man in his mid-thirties visited the Andrology outpatient clinic at Dr. Soetomo General Hospital accompanied by his wife. They have been married for eight years and have had regular unprotected sexual intercourse, but pregnancy has never been achieved. The patient's semen analyses showed azoospermia in three examinations conducted at different times. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were significantly increased, and genetic screening showed microdeletion in the Azoospermia Factor b (AZFb) subregion. Y-chromosome microdeletion is the second most common genetic defect causing azoospermia after Klinefelter syndrome. The AZF region in the distal part of the Y chromosome plays a key role in regulating spermatogenesis. Mutation or loss of any subregions in this factor may affect spermatogenesis, with the worst outcome being azoospermia. Detailed examinations are important to determine the cause of azoospermia, which may assist a physician in choosing the appropriate management for this condition. Infertile men with Y-chromosome microdeletion face challenges in reproducing naturally. They may also need genetic counseling regarding the possibility of passing on this genetic defect to their offspring and information on how to prevent it.

1. Introduction

Infertility is defined as the inability of couples in their reproductive age to achieve pregnancy after 12 months of regular unprotected sexual intercourse.¹ This condition has been one of the major health problems worldwide, affecting at least one-sixth of couples in their reproductive age. Previous data have indicated that female factors contribute to at least half of infertility cases. It is now known that male factors have also contributed to the other half of all reported infertility cases, with 20% of them being combination factors of both couples.² Azoospermia is one of the many reproductive abnormalities causing male infertility. It is a condition characterized by the absence of spermatozoa in an ejaculate after centrifugation of the semen sample at 3000x g for 15 minutes.³ The diagnosis can be established after at least two semen analyses are conducted at two different times due to intraindividual variation in semen parameters. Several studies have reported that one semen analysis is sufficient to diagnose male infertility, but two semen analyses are necessary to characterize infertility.^{4,5}

Non-obstructive azoospermia (NOA) is the most severe clinical form of male infertility, affecting at least 10% of infertile men, and Y-chromosome microdeletion has been detected in about 13% of men with NOA. It can also be found in 5% of men with severe oligozoospermia.^{6,7} The long arm in the Y chromosome consists of three known azoospermia factor (AZF) subregions: (1) AZFa in the proximal segment, (2) AZFb in the medial segment, and (3) AZFc in the distal segment. The AZF region is highly susceptible to deletions that cause spermatogenic failure and is usually associated with various histological findings in the testes, ranging from Sertoli-cell only syndrome (SCOS), maturation arrest, to hypospermatogenesis.⁸

Until recently, the gold standard management of men with Y-chromosome microdeletion is microdissection testicular sperm extraction (mTESE) followed by intracytoplasmic sperm injection (ICSI). It has been reported that TESE has a success rate of 52% in retrieving the sperm cells necessary for ICSI in men with NOA. However, the success rate of mTESE in men with NOA caused by Y-chromosome microdeletion may vary, depending on the AZF subregion affected by the deletion.⁹ Men with Y-chromosome microdeletion who wish to father a child will need genetic counseling and will be required to undergo pre-implantation genetic testing (PGT) regarding the possibility of passing on the genetic defects to their offspring. In several cases where the success rate of

mTESE is extremely low, however, adopting a child may be considered as a choice.³

The discussion of male infertility cases with non-obstructive azoospermia due to Y-chromosome microdeletion aims to increase public awareness, especially among healthcare professionals, regarding this condition. Additionally, a deeper understanding of the genetic mechanisms and potential future interventions is needed to improve the quality of life and reproductive options for those affected by this condition.

2. Case

A man in his mid-thirties visited the Andrology outpatient clinic at Dr. Soetomo General Hospital accompanied by his wife with the intention of having a child. They have been married for eight years and have had regular unprotected sexual intercourse, but pregnancy has never been achieved. It was the first marriage for the patient but was the second for his wife, who had a 16-year-old daughter from the previous marriage. The patient stated that since the beginning of their marriage, they have not been delaying pregnancy. He also stated that his libido was normal. The patient and his wife have had foreplay before every attempted sexual intercourse. The patient was able to achieve orgasm and ejaculated 5-10 minutes after penetration. Post-coital reflux was reported, and there were no reports of dyspareunia. The patient reported the onset of puberty at the age of 13. Besides having sexual intercourse, the patient had also masturbated several times from his teenage years until now, especially when his wife was on her period. The patient had a history of smoking a pack of cigarettes a day for 20 years. History of pain and trauma in the genital area, scrotal or pelvic surgery, sexually transmitted disease, hernia, varicocele, congenital abnormalities, mumps, and other chronic diseases were all denied. The patient reported never taking any medications associated with fertility issues. The patient stated that no family members had experienced similar fertility issues.

The patient's wife had a history of regular menstruation, with a cycle of 28-30 days, which lasted for 5-7 days. In 2017, the patient's wife recalled that she didn't have her period for two months. At the time, she had herself tested by using a test pack, which showed a positive result. Around two weeks later, she experienced bleeding. Afterward, she had herself tested again by using a test pack, which showed a negative result. Although the patient was aware of the situation, none of the test results were confirmed by an

Obstetrician. It remains unclear whether the patient's wife was pregnant or missing her period because of stress due to her workload. In 2023, the patient and his wife decided to undergo a fertility check-up with an Obstetrician and Gynecologist (OB-GYN) at Islamic Hospital Surabaya. The patient's wife's ultrasonography and hysterosalpingography results showed an obstruction in both of her fallopian tubes, bilateral hydrosalpinx, and a fairly large ovarian cyst. The OB-GYN advised her to undergo salpingostomy and laparotomy cystectomy. While his wife was undergoing treatment, the patient was also advised to undergo semen analysis. The first semen analysis at Biotest Klinik Utama indicated azoospermia. The patient then underwent a testicular ultrasound (Figure 1) at Islamic Hospital Surabaya, which concluded that the patient had bilateral small testes (right testis 0.74 ml; left testis 0.46 ml).

The patient and his wife were referred to the Urology outpatient clinic at Dr. Soetomo General Hospital for further examination. In January 2024, the Urologist asked the patient to undergo a hormonal (FSH, LH, and testosterone) test. Upon receiving the results, which showed a **significant increase in FSH and LH levels**, the patient was advised to undergo a testicular biopsy, but he refused. The patient and his wife visited the Andrology outpatient clinic at Dr. Soetomo

General Hospital in February 2024 for further examination following a referral from the Urologist. The patient's physical examination is shown in Table 1.

The patient underwent another semen analysis in the Andrology outpatient clinic, which showed **azoospermia** (Table 2). He also underwent another hormonal (FSH, LH, total testosterone, and estradiol) test and genetic screening for Y-chromosome microdeletion. Karyotyping was not performed on the patient due to financial limitations. The hormonal test of FSH, LH, and testosterone did not differ significantly from the first result (Table 3). Genetic screening revealed a **Y-chromosome microdeletion in the AZFb subregion (sY134)**.

Based on bilaterally low testicular volume with a soft consistency on physical examination, semen analysis showing azoospermia with normal volume and pH, high levels of FSH and LH, as well as Y-chromosome microdeletion in AZFb subregion, it can be concluded that the patient had NOA that cause primary male infertility.

The patient was given information regarding his examination results and the possible etiology associated with Y-chromosome microdeletion. He was also given information regarding the chance of natural conception, mTESE, ICSI, the possibility of passing on his genetic defect to his offspring, and the likelihood of adopting a child.

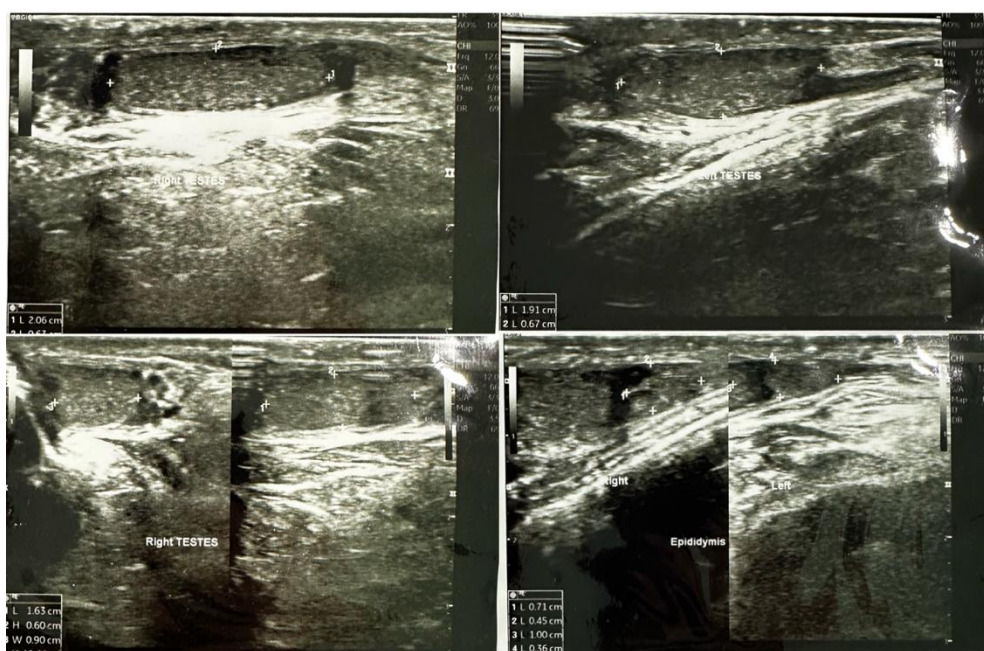


Figure 1. The Patient's Testicular Ultrasound

Table 1. The Patient's Physical Examination

General condition	:	Good
Alertness	:	Compos mentis
Blood pressure (BP)	:	110/70 mmHg
Heart rate (HR)	:	80 times/min.

Respiratory rate (RR)	:	20 times/min.
Temperature	:	Afebrile
Height	:	173 cm
Weight	:	79 kg
Body mass index (BMI)	:	26.4 kg/m² (obesity class I; Asia-Pacific classification)
Upper body length (UBL)	:	88 cm
Arm span	:	176 cm
Waist circumference	:	88 cm
Eye	:	Normal visual field
Nose	:	Hyposmia (-), anosmia (-)
Neck	:	Lymph node enlargement (-)
Chest	:	Normal S1/S2, murmur (-), gallop (-), ronchi (-)
Gynecomastia	:	None
Lipomastia	:	None
Abdomen	:	Mass (-), tenderness (-)
Inguinal	:	Hernia (-), no gonads were palpable
Extremity	:	Varus (-), valgus (-)
External genitalia		
Penis	:	Normal
Penile circumference	:	9.5 cm
Flaccid penile length (FPL)	:	8.5 cm
Stretched penile length (SPL)	:	11.5 cm
Urethral meatus	:	Normal
Urethral discharge	:	None
Scrotum	:	Normal
Hernia/Hydrocele/Dermatitis	:	None
Pubic hair	:	Thick, Tanner stage V
Pubic hair pattern	:	Male pattern
Cremasteric reflex	:	Positive
Testes		
Position	:	Scrotal / Scrotal
Volume	:	3 ml / 3 ml
Consistency	:	Soft / Soft
Pain	:	- / -
Epididymis		
Size	:	Normal / Normal
Consistency	:	Firm / Firm
Pain	:	- / -
Surface	:	Flat / Flat
Vas Deferens	:	Normal / Normal
Spermatic cord	:	Normal / Normal
Varicocele	:	- / -

Table 2. The Patient’s Semen Analyses Results

Parameters	27 February 2024	25 March 2024	References (WHO) ⁹
Ejaculation abstinence	: 4 days	6 days	2 – 7 days
Sampling method	: Masturbation	Masturbation	
Wholeness of sample	: Complete	Complete	
Macroscopic			

Odor	:	Typical	Typical	Typical
Color	:	Pearly white	Pearly white	Pearly white
Viscosity	:	< 2 cm	< 2 cm	< 2 cm
Liquifaction time	:	20 mins.	25 mins.	< 60 mins.
pH	:	7.2	7.2	7.2 – 8.0
Ejaculate volume	:	1.4 mL	1.7 mL	> 1.4 mL
Microscopic				
Sperm count	:	0 / HPF	0 / HPF	
Sperm concentration	:	0	0	≥ 16 millions/mL
Total sperm count	:	0	0	≥ 39 millions/mL
Motility				
Progressive	:	-	-	≥ 30%
Non-progressive	:	-	-	1%
Immotile	:	-	-	20%
Total motility	:	-	-	≥ 42%
Morphology				
Normal morphology	:	-	-	-

Table 3. The Patient's Hormonal Test Results

Parameters	06 February 2024	29 February 2024	References
Testosterone	5.01	4.67	2.2 – 10.5 ng/mL
FSH	44.8	45.0	1.5 – 11.8 mIU/mL
LH	41.5	44.0	1.1 – 25 mIU/mL
Estradiol		26.9	< 62.1 pg/mL

3. Discussion

Infertility is one of the major health problems, affecting at least 15% of couples worldwide, with male infertility known to contribute to at least 50% of all the reported cases. Infertility itself is defined as the inability of couples in their reproductive age to conceive after 12 months of regular unprotected sexual intercourse.¹¹ In the case presented, infertility can be diagnosed as the patient and his wife have been married for eight years and have had regular unprotected sexual intercourse, but pregnancy has never been achieved. During the physical examination, it was found that the patient had bilateral small testes, each with a volume of 3 mL and soft consistency. Semen analysis indicated azoospermia, supported by significantly elevated FSH and LH levels in laboratory tests. According to available data, around 60% of azoospermia cases fall under the NOA type.¹² Non-obstructive azoospermia can be differentiated from OA through comprehensive assessments encompassing medical history, physical examination, semen analysis, hormonal evaluation, and genetic

screening. Genetic screening is recommended for infertile males as Y-chromosome microdeletion is the second most common genetic abnormality associated with NOA, after Klinefelter syndrome.^{3,7,13}

The medical history of cryptorchidism, mumps orchitis, testicular trauma or torsion, radiation, chemotherapy, anabolic steroid usage, and brain surgery is pertinent for identifying potential etiological factors for NOA. Conversely, a medical history that includes hernia repair, scrotal or pelvic surgery, vasectomy, and genitourinary infection may contribute to OA. The volume and consistency of the testes, together with the evaluation of the vas deferens and the attributes of the epididymis, must be examined. The testes of men with OA exhibit a firm texture. In contrast, men with NOA typically possess testicles less than 15 mL. Observable testicular abnormalities necessitate further assessment by imaging techniques, as azoospermic men, especially those with NOA, face a heightened risk of testicular cancer. A healthy epididymis is firm, while an obstructed epididymis is pliable. Patients with NOA generally exhibit palpable vas deferens and flat epididymis. Ejaculates from men

with NOA typically display normal volume and pH, suggesting functional seminal vesicles and unobstructed ejaculatory ducts. In contrast, ejaculate volume less than 1.5 mL is characteristic in patients with hypogonadotropic hypogonadism NOA. An ejaculate of less than 1.5 mL, with an acidic pH (< 7.2) and reduced fructose levels, may be associated with OA; the former pertains to the congenital bilateral absence of vas deferens (CBAVD), while the latter relates to ejaculatory duct obstruction.³ In this case, biochemical analysis of semen was not performed based on the semen analysis result showing normal volume and pH.

The pituitary gland produces FSH and LH, which play roles as the primary endocrine stimuli involved in spermatogenesis. Specifically, FSH targets Sertoli cells within the seminiferous tubules, initiating their proliferation, maturation, and regulation. Meanwhile, LH primarily targets Leydig cells in the interstitial space, stimulating them to produce testosterone. In men with normozoospermia, the normal range of FSH and LH falls between 2–7 IU/L and 1.8–8.6 IU/L, respectively. Testosterone is the primary sex hormone and an anabolic steroid that has various roles in the development of reproductive system tissues and the appearance of secondary sexual characteristics in men. It also plays a key role in regulating spermatogenesis in the testes. Specifically, testosterone is produced by Leydig cells in the interstitial space of the testes as a response to LH stimulation and acts as a paracrine factor that diffuses into the seminiferous tubules. Androgen receptors found in Sertoli cells are known to play an important role in spermatogenesis.

Variations and abnormal expression of these receptors have been associated with the occurrence of NOA. Normal testosterone levels in adult males range between 240–950 ng/mL. In contrast to FSH and LH, testosterone levels decrease as age increases by about 1–2% each year. Compared to fertile males, men with NOA tend to have lower or possibly comparable testosterone levels. Elevated FSH levels above 7.6 IU/L and normal or decreased total testosterone levels in men with bilateral testicular atrophy may indicate primary testicular failure.¹⁴

In the case presented, the patient had normal testosterone and estradiol levels. In the testes, testosterone is aromatized into estrogen with the help of cytochrome P450 aromatase. Several studies have reported that estrogen has various effects on testicular development, germ cell

apoptosis, sperm cell activation, and spermatogenesis by binding to the estrogen receptors within the cell or on the cell membrane to induce genetic and non-genetic signal transductions. Estradiol is known to inhibit testosterone synthesis in Leydig cells by suppressing LH secretion. Elevated estradiol levels can be found in infertile males with oligozoospermia or azoospermia. Although estrogen is needed in spermatogenesis, it may have a deleterious effect on spermatogenesis, sperm maturation, and male fertility.¹⁵

The patient underwent Y-chromosome microdeletion screening, which showed the result of microdeletion in the AZFb subregion (sY134). Due to financial limitations, he did not undergo karyotyping or a testicular biopsy, both of which are essential for confirming NOA. The AZF region is situated on the long arm of the Y chromosome, precisely at Yq11.23. It is additionally categorized into several subregions, namely AZFa, AZFb, and AZFc. The AZFa in proximal Yq11 comprises fifteen genes, including DDX3Y, UTY, USP9Y, TTY15, and eleven pseudogenes. The AZFb in the middle area of Yq11 comprises 132 genes, including RBMY, PRY, HSFY, EIF1AY, KDM5D, CDY2A, RPS4Y2, and XKRY. The AZFc is located distal to AZFb and represents the most often deleted area in infertile males. This subregion encompasses 97 genes, including DAZ, BPY, CDY1, and CSPG4LY. Spermatogenesis is known to be regulated by DDX3Y, USP9Y, RBMY, BPY2, CDY, and DAZ genes located in the AZF region.¹⁶ While complete AZF deletions are solely present in infertile males, partial AZFc deletions are prevalent in both fertile and infertile males, with a larger incidence in the latter group.¹⁷

AZFb microdeletion is more common than AZFa microdeletion, with an incidence rate of 11% of all microdeletion cases. This subregion consists of several genes associated with ribonucleic acid (RNA) splicing during spermatogenesis.¹⁸ The diagnostic test considered the gold standard for diagnosing NOA is a testicular biopsy. This procedure can be used to predict the success rate of testicular sperm extraction. Testicular biopsy results may show the following histological features: (1) hypospermatogenesis, (2) germ cell maturation arrest, (3) SCOS, (4) tubular sclerosis, or (5) combination of all these features.¹⁹ Alongside testicular biopsy, Y-chromosome microdeletion screening may be provided to men presenting with azoospermia or severe

oligozoospermia, characterized by a sperm concentration of less than 2 million/mL. Deletions are infrequently observed in infertile men with sperm concentrations ranging from 2 to 5 million/mL. The existing European clinical guidelines continue to specify a threshold sperm concentration of less than 5 million/mL. Yet, the cost-effectiveness of the test has been scrutinized in males with sperm concentrations exceeding 1 million/mL. It is essential to underscore that screening azoospermic men for Y-chromosome microdeletion is universally recommended, as it offers both a diagnosis and a reliable assessment of the success rate of mTESE.^{20,21}

The European Association of Andrology and the European Molecular Genetics Quality Network recommend that the basic primers used to detect AZFb microdeletions are sY127 and sY134. Deletions of both primers in a specific subregion are highly indicative that the person has a complete deletion. However, to differentiate between partial and complete AZFb microdeletions, extended genetic screening using primers sY105, sY121, sY1224, sY143, sY1192, and sY153 is required.²² Recent investigations, however, have indicated that primer sY1192 alone can differentiate between complete and partial AZFb deletions, hence offering a dependable prognosis of mTESE. The variability in the breakpoint of AZFb microdeletions may result in the retention of the sY1224 proximal border marker; therefore, it is advisable to utilize the primer sY121 followed by sY1224 if the former is lacking.²⁰

Microdissection testicular sperm extraction (mTESE) is considered the gold standard for treating men with NOA, and Y-chromosome microdeletion screening has been considered the best clinical predictor for sperm retrieval through mTESE in men with NOA. If the result of the genetic screening shows complete deletion in the AZFa or AZFb subregion, sperm retrieval through mTESE is not recommended. In addition to Y-chromosome microdeletion screening, several studies suggest that testicular histology can help predict the prognosis for sperm retrieval. Hypospermatogenesis, commonly found in AZFc microdeletion, is characterized by the presence of a decreasing number of all stages of cells during spermatogenesis. Men with this histological feature have the highest chance of sperm retrieval through mTESE (50-60%), which makes it possible for them to father a child through ICSI. However, genetic counseling is necessary to inform them of the risk of passing the genetic

defect to male offspring. Several studies have reported that although men with this histological feature may conceive naturally, some of their male offspring may be azoospermic. In contrast to hypospermatogenesis, men with AZFa or AZFb microdeletion showing SCOS or maturation arrest histological features, respectively, have lower chances of sperm retrieval. The histological feature of SCOS is characterized by the absence of germ cells, with only Sertoli cells lining the seminiferous tubules. Maturation arrest is marked by halted spermatogenesis, particularly at the pre-meiotic or spermatocyte stage.^{23,24}

The limitation of this case is that the patient did not undergo the extended screening to determine whether he had a partial or complete deletion of the AZFb subregion. Although the average sperm retrieval rate in men with complete deletion of AZFb subregion was reported to be 0%, a study conducted by Zhang et al. reported that 3 of 11 men with AZFb microdeletion who underwent mTESE had a positive sperm retrieval, and 2 of them resulted in pregnancy. However, it remains unclear whether these three men had partial or complete AZFb subregion deletions.^{18,25}

4. Conclusion

The infertility experienced by the patient in this case is caused by NOA due to Y-chromosome microdeletion. Azoospermia caused by Y-chromosome microdeletion is a genetic condition that impairs spermatogenesis. An accurate diagnosis is important for the patient to receive treatment options suitable for his condition. Men with AZFb microdeletions face significant challenges in reproducing naturally because, in most cases, sperm cells cannot be found in the ejaculate due to germ cell maturation arrest. In cases of partial AZFb microdeletion, the mTESE procedure may be considered to retrieve sperm cells that can be used for ICSI. However, the sperm retrieval rate in men with AZFb microdeletions remains very low; thus, adoption may be considered for couples desiring children. Genetic counseling should also be provided to men with Y-chromosome microdeletions undergoing mTESE and ICSI procedures regarding the possibility of passing on this genetic abnormality to their male offspring.

Author's Contribution

All authors have contributed to the final manuscript. Writing – original draft preparation, J.A., S.S., and M.C.H., writing – review and

editing, W.W., supervision – A.A. and P.N. All authors have read and agreed to the published version of the manuscript.

Acknowledgement

We want to thank the Andrology outpatient clinic in Dr. Soetomo General Hospital for supporting this study.

Conflict of Interest

The authors declare no conflict of interest.

Funding Disclosure

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology*. 2015 Dec 12;13(1).
2. Agarwal A, Majzoub A, Parekh N, Henkel R. A schematic overview of the current status of male infertility practice. Vol. 37, *World Journal of Men's Health*. Korean Society for Sexual Medicine and Andrology; 2019.
3. Andrade DL, Viana MC, Esteves SC. Differential diagnosis of azoospermia in men with infertility. Vol. 10, *Journal of Clinical Medicine*. MDPI; 2021.
4. Chiu YH, Edifor R, Rosner BA, Nassan FL, Gaskins AJ, Mínguez-Alarcón L, et al. What Does a Single Semen Sample Tell You? Implications for Male Factor Infertility Research. *Am J Epidemiol*. 2017 Oct 15;186(8):918–26.
5. Leushuis E, Van Der Steeg JW, Steures P, Repping S, Bossuyt PMM, Blankenstein MA, et al. Reproducibility and reliability of repeated semen analyses in male partners of subfertile couples. *Fertil Steril*. 2010 Dec;94(7):2631–5.
6. Cerván-Martín M, Castilla JA, Palomino-Morales RJ, Carmona FD. Genetic landscape of nonobstructive azoospermia and new perspectives for the clinic. Vol. 9, *Journal of Clinical Medicine*. MDPI; 2020.
7. Pandiyan N, Khan SD. A clinical approach to male infertility. In: *Male Infertility: A Clinical Approach*. Springer India; 2016. p. 41–54.
8. Chen SW, Chen CP, Chern SR, Kuo YL, Chiu CL. The significance of karyotyping and azoospermia factor analysis in patients with nonobstructive azoospermia or oligozoospermia. *Taiwan J Obstet Gynecol*. 2022 Sep 1;61(5):800–5.
9. Sha J, Huang G, Zhang B, Wang X, Xu Z, Zhai J. Chromosomal abnormalities and Y chromosome microdeletions in infertile men with azoospermia and oligozoospermia in Eastern China. *Journal of International Medical Research*. 2019;48(4).
10. WHO. WHO laboratory manual for the examination and processing of human semen Sixth Edition. 2021.
11. Arumugam M, Shetty DP, Kadandale JS, Kumari SN. Y chromosome microdeletion and cytogenetic findings in male infertility: A cross-sectional descriptive study. *Int J Reprod Biomed*. 2021;19(2):147–56.
12. Cioppi F, Rosta V, Krausz C. Genetics of azoospermia. Vol. 22, *International Journal of Molecular Sciences*. MDPI AG; 2021.
13. Chabchoub I, Kdous M, Zhioua F, Gaied A, Merdassi G. Y chromosome microdeletions screening in Tunisian infertile men. *Ann Biol Clin (Paris)*. 2019;77(5):517–23.
14. Tao Y. Endocrine aberrations of human nonobstructive azoospermia. Vol. 24, *Asian Journal of Andrology*. Wolters Kluwer Medknow Publications; 2022. p. 274–86.
15. Luo H, Huang Y, Han M, Pang Y, Yu P, Tang Y, et al. Associations of serum estradiol level, serum estrogen receptor-Alpha level, and estrogen receptor-Alpha polymorphism with male infertility: A retrospective study. *Medicine (United States)*. 2021 Jul 23;100(29):E26577.
16. Colaco S, Modi D. Consequences of Y chromosome microdeletions beyond male infertility. Vol. 36, *Journal of Assisted Reproduction and Genetics*. Springer New York LLC; 2019. p. 1329–37.
17. Bansal SK, Jaiswal D, Gupta N, Singh K, Dada R, Sankhwar SN, et al. Gr/gr deletions on Y-chromosome correlate with male infertility: An original study, meta-analyses, and trial sequential analyses. *Sci Rep*. 2016 Feb 15;6.

18. Yuen W, Golin AP, Flannigan R, Schlegel PN. Histology and sperm retrieval among men with y chromosome microdeletions. Vol. 10, *Translational Andrology and Urology*. AME Publishing Company; 2021. p. 1442–56.
19. Esteves SC. Clinical management of infertile men with nonobstructive azoospermia. Vol. 17, *Asian Journal of Andrology*. Wolters Kluwer Medknow Publications; 2015. p. 459–70.
20. Krausz C, Navarro-Costa P, Wilke M, Tüttelmann F. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: State of the art 2023. Vol. 12, *Andrology*. John Wiley and Sons Inc; 2024. p. 487–504.