

REVIEW ARTICLE

Role of Genetic Testing in Male Infertility**Ponco Birowo****Department of Urology, Faculty of Medicine, Universitas Indonesia/
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Abstract

Male-factor infertility is responsible for 30-55% of all infertility cases. The causes of male infertility include varicocele, endocrine disorders, genital tract infections, genetic disorders and idiopathic. It is estimated that genetic abnormalities contribute to 50% of male infertility. In daily practice, the diagnosis of male infertility has been based on history taking, relevant physical examination, hormone tests and basic semen analysis with a strong emphasis on the assessment of sperm concentration, motility, and morphology. Although recent development in assisted-reproductive technologies such as in vitro fertilization and intrauterine insemination increases the chance of clinical pregnancy and live birth, genetic counseling and testing should always be performed whenever genetic risks are related to the cause of infertility for the identification of possible genetic abnormalities and to assess the risk of transmitting the genetic defects to future generations. Genetic defects affect male infertility by disrupting hormonal homeostasis, spermatogenesis, and sperm quality. These genetic defects include chromosomal abnormalities (e.g. Klinefelter Syndrome), Y chromosome deletions, and cystic fibrosis transmembrane conductance regulator gene mutations. The utilization of genetic counseling and testing is also important to predict the success of sperm retrieval in men with certain genetic abnormalities. To name a few, genetic analysis at the chromosomal level (karyotyping), androgen receptor gene mutations test, cystic fibrosis test, and Y chromosome microdeletions analysis should be considered in the diagnosis of male factor infertility where genetic risks are present.

Keywords: male infertility; genetic disorders; genetic testing; genetic counseling; chromosomal abnormalities; karyotyping

Peran Pemeriksaan Genetik pada Infertilitas Laki-laki**Abstrak**

Infertilitas yang disebabkan oleh faktor laki-laki sebanyak 30-55% dari semua kasus infertilitas. Penyebab infertilitas antara lain varikokel, kelainan endokrin, infeksi saluran kemih, kelainan genetik dan idiopatik. Kelainan genetik diperkirakan 50% dari seluruh kasus infertilitas laki-laki. Pada praktik sehari-hari, diagnosis infertilitas laki-laki didasarkan pada anamnesis, pemeriksaan fisik yang relevan, pemeriksaan hormon dan analisis sperma dasar yang diutamakan pada konsentrasi sperma, motilitas, dan morfologi. Perkembangan terkini teknologi reproduksi berbantuan seperti fertilisasi in vitro dan inseminasi intrauterin meningkatkan kemungkinan keberhasilan kehamilan dan memiliki anak, namun konseling dan pemeriksaan genetik harus selalu dikerjakan jika risiko genetik berhubungan dengan penyebab infertilitas. Tujuannya adalah untuk mengidentifikasi kemungkinan kelainan genetik dan menilai risiko defek genetik tersebut menurun ke generasi selanjutnya. Defek genetik mempengaruhi infertilitas laki-laki dengan merusak homeostasis hormon, spermatogenesis, dan kualitas sperma. Defek tersebut termasuk kelainan kromosom (Sindrom Klinefelter), delesi kromosom Y, dan mutasi gen fibrosis transmembrane conductance regulator. Pemanfaatan konseling dan pemeriksaan genetik juga penting dalam memprediksi tingkat kesuksesan pengambilan sperma pada laki-laki dengan kelainan genetik tertentu. Analisis genetik pada tingkat kromosom (karyotyping), pemeriksaan mutasi gen reseptor androgen, pemeriksaan cystic fibrosis, dan analisis mikrolelesi kromosom Y dapat dipertimbangkan dalam diagnosis infertilitas yang disebabkan oleh faktor laki-laki yang memiliki risiko genetik.

Kata kunci: infertilitas laki-laki; kelainan geneti; pemeriksaan genetik; konseling genetik; kelainan kromosom; karyotyping

Introduction

Infertility is defined as the inability of a sexually active couple to conceive after 12 months of unprotected, regular intercourse.¹ This condition affects approximately 15% of couples worldwide and involves both men and women factors.² Male-factor infertility is responsible for 30-50% of all infertility cases, either solely or in combination with female factors.^{3,4} Male infertility is caused by a wide variety of disorders include varicocele, endocrine disorders, genital tract infections, genetic disorders, and idiopathic origin.⁴

Azoospermia, the absence of spermatozoa in the ejaculate after at least two assessments of centrifuged semen, is found in 10-15% of all male infertility cases.⁵ Based on the patency of the sperm release pathway, azoospermia is divided into two categories: obstructive azoospermia (OA) and non-obstructive azoospermia (NOA).⁶ OA results from obstruction of the excurrent duct at any location between the rete testis and the ejaculatory duct. This condition contributes to 40% of all azoospermia cases.⁶ NOA is the most common form of azoospermia and it is caused by conditions other than hypothalamic-pituitary disease and obstruction of the male genital tract.⁷

Genetic disorders account for 15-30% of azoospermia,⁸ whereas idiopathic male infertility, in which there are normal findings on physical examination and endocrine, genetic and biochemical laboratory testing, is found in 12-41% of all azoospermia cases and it is likely that most of this case has underlying genetic causes.⁴ Common genetic disorders that may cause male infertility include chromosomal abnormalities (Klinefelter Syndrome/ KS, Robertsonian translocation), Y chromosome deletions, and cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation.⁹

The invention of assisted reproductive technology (ART) has increased the chance of men with azoospermia to have offspring. Methods of sperm retrieval such as percutaneous epididymal sperm aspiration (PESA) and testicular sperm extraction (TESE) enables clinicians to retrieve

sperms directly from the epididymis or the testicles of men with azoospermia to be used in in vitro fertilization (IVF) or intrauterine insemination (IUI). However, although the development of ART increases the chance of sperm retrieval among men with azoospermia, it does not prevent the transmission of genetic defects to the offspring. Children of men with Y chromosome deletions, for example, can inherit the defects as well as having an increased chance of having whole Y chromosome deletions.¹⁰ Therefore, in addition to the basic diagnostic strategy for male infertility, which often comprises of history taking, physical examination, endocrine testing, semen analysis, and imaging studies, further diagnostic investigation involving genetic counseling and testing is needed not only for diagnostic purpose, but also to make a prognosis of sperm retrieval result and to take necessary precautions to prevent the transmission of genetic defects to offspring.¹¹

Genetic Abnormalities Related to Male Infertility

Genetic abnormalities associated with male infertility could be manifested in both OA and NOA phenotypes (Table 1). However, men with certain genetic defects can also produce sperm, although, with aneuploidies. The availability of IVF and IUI techniques increases the chance of transmitting this genetic defects to the future generations. Furthermore, children conceived via ART have an increased risk of having abnormal karyotype with approximately half of them are fathered by men with chromosomal abnormalities.⁹ In this paper, we mainly discuss three genetic defects that have pathological effects towards male fertility namely KS, Y chromosome deletions, and CFTR gene mutations. Chromosomal defects contribute as the most frequent genetic abnormalities affecting male infertility. It is detected in approximately 20% of men with abnormal semen parameters.¹² The most common type of constitutional chromosome aberration among infertile men is represented by KS, while the most frequent structural chromosomal abnormality is Y chromosome microdeletions.¹³

Table 1. Prevalence of Common Genetic Abnormalities Associated with Male Infertility⁹

Genetic Abnormality	Prevalence
Chromosomal Aberrations	
Klinefelter syndrome	5-10% azoospermia; 2-5% severe oligozoospermia
Robertsonian translocations	0.5-1%
Reciprocal translocations	0.5-1%
Y Chromosome Deletions	
AZFa	0.5-1%
AZFb	0.5-1%
AZFc	3-7%
AZFb+c	0.5-1%
Gene Mutations	
CFTR	60-70% (5% in infertile men)
AR	2-3%

AZF = azoospermia factor, CFTR = cystic fibrosis transmembrane conductance regulator, AR = androgen receptor

Klinefelter Syndrome

KS is the most common genetic cause of male hypogonadism. Approximately 1 in 600 newborn males is affected by KS.¹⁴ The term KS originated from Harry F. Klinefelter, who in 1942 described for the first time a clinical entity of men with gynecomastia, small testes, absent spermatogenesis, normal to moderate reduction of Leydig cell function and an increased level of FSH.¹⁵ KS is caused by a supernumerary X chromosome with 80% of men with KS are of the non-mosaic 47,XXY karyotype, while the other 20% are of the 46,XY/47,XXY mosaicism.¹⁶ Ferlin et al⁹ reported that 76.6% of non-mosaic KS had complete azoospermia, while among the mosaic 46,XY/47,XXY men, 74.4% were azoospermic.

The prognosis of men with KS to father children before the introduction of IVF was not favourable. Now, however, men with KS have an increased chance of having children with the help of surgical sperm retrieval techniques.¹⁷ The sperm of KS men usually have a normal 23,X or 23,Y haploid genome and most of their children are chromosomally normal. However, an increased rate of autosomal and sex chromosome aneuploidies was reported in KS men's offspring. This means there is a significant risk of these children to father their own offspring with 47,XXY or 47,XXX karyotype.^{18,19}

Although the classical signs and symptoms of KS are recognized, many patients with KS only present with obscure phenotype. Therefore, the abnormality is underdiagnosed. It is estimated that only one-fourth of men with KS receive diagnoses.¹⁴ Consequently, with the development

of advanced sperm retrieval techniques widely available nowadays, the rate of transmission of the disease towards future generations also increases.

Boys with KS usually show some symptoms such as speech development delay, learning disabilities, or behavioral problems.²⁰ Increased height velocity rate is usually notable between the age of 5 to 8, but differentiating between boys with KS and normal boys based on the physical appearance is difficult as the magnitude or timing of pubertal growth spurt does not differ significantly between boys with KS and normal boys.²¹ The abnormality is usually diagnosed after puberty, when men with KS start noticing small testicular size and symptoms of androgen deficiency with most of the patients diagnosed when they are unable to conceive and sperm analysis shows azoospermia.²⁰ Early recognition of the signs and symptoms of KS, together with confirmation by karyotyping are of vital value in the diagnosis of KS.

Y Chromosome Deletions

Y chromosome deletions are the second most common genetic abnormalities affecting male infertility after KS. It is estimated that the prevalence of Y chromosome deletions is around 1:2000 to 1:3000 men.^{22,23} The azoospermia factor AZF located on the Y chromosome is divided into three regions: AZFa, AZFb, and AZFc based on their location from proximal to the distal of the chromosome.²⁴ These regions contain genes that regulate germ cell development and involved in the pathophysiology of male infertility, i.e. azoospermia and severe oligozoospermia.²⁵

The incidence of Y chromosome microdeletions ranges from 1 to 55%.^{26,27} AZFc deletions contribute as the most common Y chromosome microdeletions (65-70%), followed by the AZFb, AZFb+c, or AZFa+b+c microdeletions (25-30%). AZFa deletions are the rarest form of Y chromosome microdeletions, comprising of 5% of all Y chromosome microdeletions.²⁸

AZFa deletions are associated with Sertoli cell-only syndrome (SCOS) and testicular histopathology shows complete germ cell loss and degeneration of seminiferous tubules. It is a result of non-allelic homologous recombination (NAHR) between repeated nearly identical DNA sequence.¹⁶

AZFb and AZFb+c deletions are also associated with SCOS or pre-meiotic spermatogenic arrest,¹⁷ while AZFc deletion leads to azoospermia or severe oligozoospermia with different spermatogenic phenotypes in the testis. Sperm in the ejaculate or the testis is found in approximately 60% of men with AZFc deletions.⁹

The availability of sperm retrieval techniques to obtain sperm from men with Y chromosome microdeletions facilitates the transmission of genetic abnormalities to their children as male offspring of men with AZF deletions also carry the deletion and will have impaired spermatogenesis in adulthood.⁹ Foresta et al²⁹ reported in 2005 that men with AZF genes deletions had a significantly reduced percentage of normal Y-bearing spermatozoa compared to men with normozoospermia ($p < 0.01$). The identification of AZF deletions in men with azoospermia or severe oligozoospermia is important because it has a prognostic value towards sperm retrieval success. Men with AZFa and AZFb deletions have no chance of having any sperm in the testis, whereas there is a chance of obtaining viable sperm in men with AZFc deletions.³⁰

CFTR Gene Mutations

Cystic fibrosis (CF) is an autosomal-recessive genetic disorder caused by mutations in CFTR gene, located on the long arm of chromosome 7. It encodes a membrane protein that serves as an ion channel and regulates the formation of the ejaculatory duct, seminal vesicle, vas deferens and distal two-thirds of the epididymis. CFTR gene mutations are found in 5% of all infertile men and in 60-70% of OA, in particular among patients with congenital bilateral absence of the vas deferens (CBAVD), making them the most common genetic-abnormality cause of OA.⁹

Other Examples of Genetic Abnormalities Affecting Male Fertility

Robertsonian and reciprocal translocations are common structural chromosomal abnormalities affecting male fertility. They contribute to 1% of all male infertility and usually show as azoospermia phenotype than oligozoospermia.⁴ Alteration of the gene expression pattern of the spermatozoa at many stages is found in Robertsonian translocations,³¹ while impaired semen parameters or sperm aneuploidies are found among men with reciprocal translocations, although the exact mechanism is still not known.⁴ In addition to KS, other numerical chromosome abnormalities with variable fertility status can also occur, such as 47,XYY and 45,X/46,XY mosaicism.⁸ Although phenotypically normal, men with 47,XYY karyotype may have spermatogenesis failure, maturation arrest, or SCOS.⁹

Androgens (testosterone and 5 α -dihydrotestosterone/DHT) are the hormones that regulate the development and maintenance of spermatogenesis.⁴ The androgen receptor (AR) is encoded by a gene located on the X chromosome and consequently, mutations in this gene cause a collection of defects known as androgen insensitivity syndrome (AIS). It is found in 2% of all infertile men and the majority of its patients may only have infertility as their sole symptoms.⁹

Genetic Counseling and Testing in Male Infertility

The diagnostic procedures to assess male infertility usually consist of history taking, physical examination, hormone levels, basic semen analysis, and relevant imaging studies. However, it is reported that routine semen analysis with emphasis on sperm concentration, motility, morphology alone is not enough in the diagnosis of male infertility.³² In large centers, genetic counseling with genetic testing of male infertility using chromosomal analysis such as karyotyping, AR gene mutations test, CF test, and Y chromosome microdeletion analysis have been employed routinely.⁴

Genetic counseling involves a discussion of possible genetic conditions affecting male fertility and its importance in the routine assessment of male infertility. A successful genetic counseling comprises of relevant information exchange, presentation of choices related to the patient's fertility status, and discussion of patient values and beliefs.³⁰ Genetic counseling among couples, particularly regarding male infertility, is of paramount importance because, after successful conceptions genetic abnormalities, e.g. Y chromosome microdeletions, are also

transmitted to the male offspring. In most cases, father and the son share the same magnitude of genetic abnormalities. However, in certain cases, the offspring has larger genetic defects and the process will continue to future generations.³³

According to the European Association of Urology (EAU), standard karyotype analysis must be performed to all men with a sperm concentration <10 million/mL who are seeking fertility treatment by IVF.³⁴ Based on the American Urological Association (AUA), karyotyping and genetic counseling should be offered to all patients with NOA and severe oligozoospermia with sperm concentration <5 million/mL.³⁵

Regarding Y chromosome microdeletions, the EAU stated that testing for microdeletions is not necessary in men with OA but should be offered to men with sperm concentration <5 million/mL for diagnostic and prognostic purpose. Men with complete AZFa or AZFb microdeletions are not advised to undergo sperm retrieval procedure as it is unlikely that any sperm will be found.³⁴ The AUA, however, stated that although the prognosis for sperm retrieval is poor for patients with AZFa or AZFb deletions, the results of Y chromosome deletion analysis are not able to absolutely predict the absence of sperm.³⁵ The EAU and AUA guidelines agree that in men with congenital bilateral absence of the vas deferens, both partners should be tested for CFTR gene mutations.^{34,35}

Conclusion

The availability of sperm retrieval techniques to obtain sperm from men with azoospermia or severe oligozoospermia enables couples who previously had little chance of having children to conceive with the help of ART techniques. However, a large portion of these men have genetic abnormalities, which affect the spermatogenesis and if sperm is produced, increase the risk of transmitting the genetic abnormalities to future generations. Nowadays, genetic causes of male infertility have been recognized, whereas genetic counseling of infertile couples has markedly improved. Appropriate counseling based on the familial and reproductive history should be offered and certain genetic testing must be offered in routine assessment of male infertility, not only for diagnostic purpose but also to make a prognosis of sperm retrieval result and to take necessary precautions to prevent or manage the transmission of genetic defects to future generations.

References

1. Nieschlag E, Behre HM, Nieschlag S. *Andrology: male reproductive health and dysfunction*. Berlin: Springer-Verlag Berlin Heidelberg; 2010.
2. de Kretser DM. Male infertility. *Lancet*. 1997;349((9054)):787–90.
3. Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. *Fertil Steril*. 2002;77(5):873–82.
4. Tahmasbpour E, Balasubramanian D, Agarwal A. A multi-faceted approach to understanding male infertility: gene mutations, molecular defects and assisted reproductive techniques (ART). *J Assist Reprod Genet*. 2014;31(9):1115–37.
5. Jarvi K, Lo K, Fischer A, Grantmyre J, Zini A, Chow V, et al. CUA guideline: the workup of azoospermic males. *J Can Urol Assoc*. 2010;4(3):163–7.
6. Jarow JP, Espeland MA, Lipshultz LI. Evaluation of the azoospermic patient. *J Urol*. 1989;142:62–5.
7. Niederberger C. WHO manual for the standardized investigation, diagnosis and management of the infertile male. Vol. 57. *Urology*. New York: Elsevier; 2001.
8. Vogt PH. Molecular genetics of human male infertility: from genes to new therapeutic perspectives. *Curr Pharm Des*. 2004;10(5):471–500.
9. Ferlin A, Arredi B, Foresta C. Genetic causes of male infertility. *Reproductive Toxicology*. New York: Elsevier; 2006.p.133–41.
10. Komori S, Kato H, Kobayashi S, Koyama K, Isojima S. Transmission of Y chromosomal microdeletions from father to son through intracytoplasmic sperm injection. *J Hum Genet*. 2002;47(9):465–8.
11. Hamada AJ, Esteves SC, Agarwal A. A comprehensive review of genetics and genetic testing in azoospermia. *Clinics (Sao Paulo)*. 2013;68 Suppl 1:39–60.
12. O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. *Fertil Steril*. 2010;93(1):1–12.
13. Foresta C, Ferlin A, Gianaroli L, Dallapiccola B. Guidelines for the appropriate use of genetic tests in infertile couples. *Eur J Hum Genet*. 2002;10:303–12.
14. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab*. 2003;88(2):622–6.
15. Klinefelter HF, Reifenstein E, Albright F. Syndrome characterized by gynecomastia, aspermatogenesis without a-leydigism and increased excretion of FSH1. *J Clin Endocrinol Metab*. 1942;2(11):615–27.
16. Zorrilla M, Yatsenko AN. The genetics of infertility: current status of the field. *Curr Genet Med Rep*. 2013;1(4): 247–60.
17. Ferlin A, Garolla A, Foresta C. Chromosome abnormalities in sperm of individuals with constitutional sex chromosomal abnormalities. *Cytogenet Genome Res*. 2005;111:310–6.

18. Hennebicq S, Pelletier R, Bergues U, Rousseaux S. Risk of trisomy 21 in offspring of patients with Klinefelter's syndrome. *Lancet*. 2001;357:2104–5.
19. Reubinoff BE, Abeliovich D, Werner M, Schenker JG, Safran A, Lewin A. A birth in non-mosaic Klinefelter's syndrome after testicular fine needle aspiration, intracytoplasmic sperm injection and preimplantation genetic diagnosis. *Hum Reprod*. 1998;13(7):1887–92.
20. Abramsky L, Chapple J. 47,XXY (Klinefelter Syndrome) and 47,XYY: estimated rates of and indication for postnatal diagnosis with implications for prenatal counselling. *Prenat Diagn*. 1997;17(4):363–8.
21. Wikström AM, Dunkel L. Klinefelter syndrome. *Best Pract Res Clin Endocrinol Metab*. 2011;25(2):239–50.
22. Asero P, La Vignera S, Lanzafame F. Genetic aspects of male infertility. *J Andrological Sci*. 2010;17(1):1–16.
23. Vogt PH, Fernandes S. Polymorphic DAZ gene family in polymorphic structure of AZFc locus: artwork or functional for human spermatogenesis? *APMIS*. 2003;111(1):115–27.
24. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet*. 1996;5(7):933–43.
25. Sertić J, Cvitković P, Myers a, Saiki RK, Stavljenić Rukavina a. Genetic markers of male infertility: Y chromosome microdeletions and cystic fibrosis transmembrane conductance gene mutations. *Croat Med J*. 2001;42(4):416–20.
26. Foresta C, Ferlin a, Garolla a, Moro E, Pistorello M, Barboux S, et al. High frequency of well-defined Y-chromosome deletions in idiopathic Sertoli cell-only syndrome. *Hum Reprod*. 1998;13(2):302–7.
27. van der Ven K, Montag M, Peschka B, Leygraaf J, Schwanitz G, Haidl G, et al. Combined cytogenetic and Y chromosome microdeletion screening in males undergoing intracytoplasmic sperm injection. *Mol Hum Reprod*. 1997;3(8):699–704.
28. Krausz C, Degl'Innocenti S. Y chromosome and male infertility: update, 2006. *Front Biosci*. 2006;11:3049–61.
29. Foresta C, Garolla A, Bartoloni L, Bettella A, Ferlin A. Genetic abnormalities among severely oligospermic men who are candidates for intracytoplasmic sperm injection. *J Clin Endocrinol Metab*. 2005;90(1):152–6.
30. Harper J, Geraedts J, Borry P, Cornel MC, Dondorp WJ, Gianaroli L, et al. Current issues in medically assisted reproduction and genetics in Europe: research, clinical practice, ethics, legal issues and policy. *Hum Reprod*. 2014;29(8):1603–9.
31. Gunel M, Cavkaytar S, Ceylaner G, Batioglu S. Azoospermia and cryptorchidism in a male with a de novo reciprocal t(Y;16) translocation. *Genet Couns*. 2008;19(3):277–80.
32. Milardi D, Grande G, Vincenzoni F, Messina I, Pontecorvi A, De Marinis L, et al. Proteomic approach in the identification of fertility pattern in seminal plasma of fertile men. *Fertil Steril*. 2012;97(1).
33. Stuppia L, Gatta V, Calabrese G, Franchi PG, Morizio E, Bombieri C, et al. A quarter of men with idiopathic oligo-azoospermia display chromosomal abnormalities and microdeletions of different types in interval 6 of Yq11. *Hum Genet*. 1998;102(5):566–70.
34. Jungwirth A, Diemer T, Dohle, GR, Kopa Z, Krausz CHT. Guidelines on male infertility. *European Association of Urology*. 2016.p.1–24.
35. Jarow JP, Sigman M, Kolettis PN, Lipshultz LL, Dale M, Nangia AK. The optimal evaluation of the infertile male: best practice statement reviewed and validity confirmed 2011 [Internet]. *American Urological Association*, 2011. Available from: <https://www.auanet.org/education/guidelines/male-infertility-d.cfm>