REVIEW

Male Genetic Evaluation in Infertility, Recurrent Abortion and Recurrent in Vitro Fertilization Failure; A Clinical Approach

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Purpose: This review presents a clinical approach to genetic issues in male infertility. Unlike other related reviews that discuss different types of genetic diseases (such as Klinefelter and Down syndrome), this review focuses on the clinical features that result from these genetic problems (such as azoospermia and oligospermia).

Methods: A narrative review of the clinical literature in PubMed was conducted using keywords related to male infertility, recurrent pregnancy loss, recurrent in vitro fertilization failure, and genetics. The search included articles with English reviews published online after 2020. Headlines were defined based on the available literature, and after a critical review of each manuscript, clinical facts were classified under the corresponding headlines, and a primary draft was written.

Results: 29 relevant articles were selected from the search. According to the literature, there are clinical genetic approaches for azoospermia, severe oligospermia, severe teratospermia, severe asthenospermia, recurrent miscarriage, and recurrent in vitro fertilization failure.

Conclusion: Although many mutations that can affect male fertility and spermogram have been identified, only a few have clinical predictive value.

Keywords: Male infertility; Genetics; recurrent pregnancy loss; recurrent in vitro fertilization failure

INTRODUCTION

Infertility, defined as the inability to conceive after one year of unprotected sexual intercourse, and unsuccessful fertility events such as abortion and failed assisted reproductive techniques (ART), are common problems affecting 10-20% of couples. The causes of infertility can be multifactorial, with both partners contributing, and rarely can a single factor be identified as the main cause[1]. Genetic causes account for about 1% of infertility cases, which may seem rare initially. However, in severe cases of infertility, such as azoospermia, severe oligospermia, absolute asthenozospermia, and absolute teratozospermia, the contribution of genetics increases to approximately 15-20%. This increased genetic role is also observed in cases of recurrent miscarriage and repeated IVF failure, although not extensively studied[2].

The importance of genetic testing extends beyond its role as a cause of infertility. It is crucial for ensuring the health of future generations. In the past, natural selection eliminated many genetic disorders that caused subfertility or infertility by preventing affected individuals from having children. However, with the advent of IVF, many of these disorders can bypass natural selection and be passed on to the next generation, especially in cases of severe disorders treated with microinjection. A gene that only manifests as infertility due to incomplete penetrance can lead to severe diseases outside of the child's reproductive system by altering its expression. Some clinicians believe that repeated assisted reproductive failures or recurrent miscarriages may be indicative of changes in the expression of silent genes in the parental generation[3].

The third point highlighting the importance of genetic testing in male fertility events is the lack of progress in the treatment of infertility, recurrent miscarriages, and repeated IVF failures over the past two decades. Scientists believe that one reason for this lack of improvement is the insufficient awareness of genetic disorders, which can potentially be addressed with the help of whole genome sequencing and artificial intelligence. Aside from the importance of genetic testing, it is crucial to bridge the gap between basic and clinical sciences. While many genetic disorders are known and sophisticated tests have been developed for them, only a few clinical tests have been widely adopted in this field. This review article aims to focus on the clinical utility and general use of these tests[4].

MATERIALS AND METHODS

A systematic online search was conducted on the PubMed database from 2020 until October 2023 using the following keywords: male infertility, genetics, recurrent IVF failure, and recurrent pregnancy loss. Based on this primary search, headlines for male infertility with a genetic approach were defined, and a secondary search was performed using these new keywords, such as azoospermia, oligospermia, etc., along with genetics. All relevant manuscripts were carefully studied, and only English-language review articles with a clinical

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Table 1. Indications of genetic testing in male infertility (10)

Disorder	Indication of genetic testing
Sperm count	$< 5 \times 10^6$/CC
Sperm motility	Absolute asthenospermia
Sperm morphology	Absolute teratospermia
miscarriage	≥ 3 miscarriages/ 2 miscarriages and more than 1 year infertility
ART failure	≥ 3 IVF or ICSI failure (either failure of embryos production or implantation failure or mix of them)

perspective were included.

Types of genetic disorders in male infertility and genetic testing

The most common genetic disorders of male infertility include defects in chromosome number, chromosome structural abnormalities, and single gene mutations. Genetic information is transferred through deoxyribonucleic acid (DNA) molecules located in the cell nucleus. Genes, which consist of a specific number and sequence of DNA molecules, are responsible for transferring phenotypes from germ cells to the next generation. Genes combine to form chromosomes[5]. Karyotyping, a process that involves staining chromosomes to reveal bands on each arm, is used to examine chromosomes. If there is a structural disorder in a chromosome with more than five million bases, the standard appearance of the chromosome bands changes, allowing for identification of the disorder. Otherwise, additional tests are needed for diagnosis. Detecting genetic structural disorders involving less than five million bases includes single gene testing, gene panels, polymerase chain reaction (PCR), and microarray. Whole genome sequencing is not currently used for evaluating male infertility. Figure 1 outlines the process for diagnosing genetic disorders contributing to male infertility[6].

Different methods are used to identify specific types of small structural genetic changes in chromosomes. For example, Y microdeletions can be detected using PCR, while microarrays are better suited for detecting copy number variations. However, urologists do not need to be familiar with these methods when making requests. They can simply request the type of disorder, and the geneticist will choose the appropriate method. Karyotyping provides general information about all chromosomes and can be requested for any type of semen analysis disorder. However, other tests are only requested if the specific phenotype of the disease is present in an infertile patient[7].

Indications of assessing male genetic causes in fertility related disorders

Clinicians often hesitate to perform genetic testing due to the frequent occurrence of normal results. However, the likelihood of identifying more genetic disorders increases as the percentage of abnormal results increases. In other words, the specificity of the test increases, leading physicians to investigate genetic causes more often[8].

Infertility causes are traditionally divided into three categories: pretesticular, testicular, and post-testicular. However, both genetic and non-genetic causes can be present in all three groups. For example, Kallman's syndrome and pituitary tumors are both pretesticular causes, while mumps orchitis and Klinefelter's syndrome are both testicular causes. Bilateral absence of vas deferens and infectious epididymitis are post-testicular causes, with one being genetic and the other non-genetic. Therefore, classifying infertility causes does not help with screening for genetic factors[9,10].

However, it has been found that the more severe the disorder is in an infertile individual, the more likely it is that genetic infertility is the cause. For example, genetic disorders account for 1% of all infertile individuals, but this increases to 15-20% in individuals with severe oligospermia, asthenospermia, teratospermia, and azoospermia[10]. Table 1 presents the indications for genetic testing.

Genetic evaluation of azoospermia and severe oligospermia

Genetic investigation for non-obstructive azoospermia and severe oligospermia is different from obstructive azoospermia. When it comes to oligo and azoospermia, there is disagreement among clinicians regarding the cutoff point for genetic testing in individuals with low sperm count. Some clinicians start genetic investigation when the number of sperm is less than ten million per milliliter. Another important consideration is the patient's history and examination. If the cause of the disorder is determined during the clinical evaluation, genetic investigation may not be necessary. For example, if a patient with azoospermia has a history of chemotherapy, genetic evaluation is not warranted[11,12].

In the case of non-obstructive azoospermia and oligospermia, it is important to note that over 2000 genes are involved in spermatogenesis. It is not necessary to examine all of these genes, only the most common ones. Y microdeletions are often associated with more severe oligospermia, and some scientists consider a sperm concentration of less than one million per milliliter as the threshold for checking microdeletion analysis. If Y microdeletion A or B is positive, there is no need for a biopsy, as sperm will not be found. On the other hand, if Y microdeletion C is present, there is a high proba-

Table 2. Genetic evaluation in severe asthenospermia

Type of oligo and azoospermia	Genetic tests	Results
Hypogonadotropic hypogonadism (Pretesticular	Panel gene screening (Not clinically approved)	Type of mutation
None-obstructive (Testicular type)	Karyotype and Y microdeletion survey	47 XXY, 47 XYY, 46 XX male, translocation and inversion Microdeletion A: Sertoli cell only syndrome Microdeletion B: Complete maturation arrest Microdeletion C: Hypo spermatogenesis
Obstructive(Post testicular)	CFTR panel gene screening in case of unpalpable vasa deferent	Positive or negative

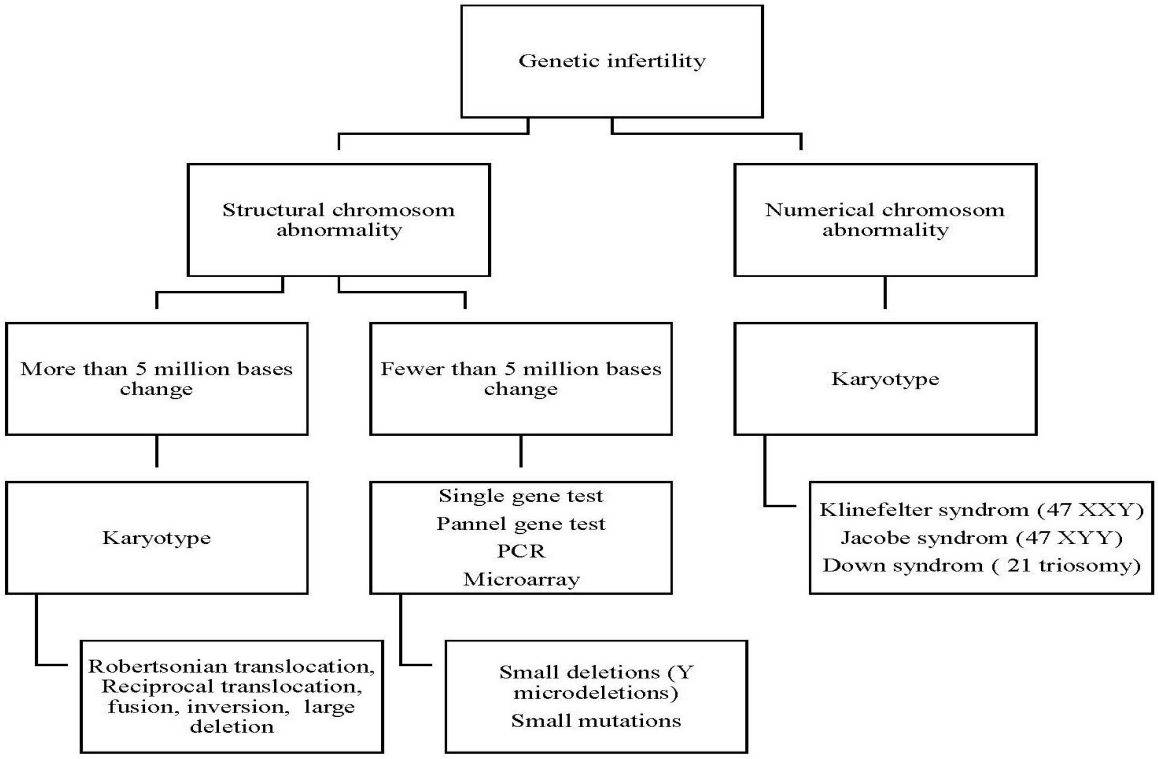

Figure 1. Genetic male infertility diagnosis

bility of finding sperm suitable for microinjection in the testicle[13].

The formation and connection of the rete testis, epididymis, and vas deferens, as well as their opening in the prostatic duct, are controlled by several genes. However, only the CFTR gene, which is associated with cystic fibrosis, pulmonary disease, and pancreatic disease, is worth investigating. Up to 80% of individuals with bilateral agenesis or hypoplasia carry the autosomal recessive CFTR gene. In unilateral cases without renal agenesis, there is a 10% chance of the gene being present. There are approximately 2000 known mutations of the CFTR gene, and panel testing typically checks for the 20-60 most common mutations. Some clinicians question the need to test this gene in affected men and only test their wives, who usually have a 2.5% chance of carrying the defective gene. Others test both partners. However, since not all mutations are checked, the risk of a child developing cystic fibrosis is not zero, but it is very low[14,15].

Hypothalamus disorders such as Kallman's syndrome are still not genetically investigated despite the availability of diagnostic tests. This is due to their low prevalence and relative treatability. However, since treatment response varies among patients, with some responding to minimal doses for a short period of time and others requiring high doses for up to two years, it has been suggested to screen for specific mutations and make decisions accordingly[16]. **Table 2** shows genetic testing in sperm count disorders.

Genetic evaluation of absolute asthenozospermia

Asthenozospermia is defined as the progressive motility of less than 32% of sperm in a semen analysis. Genetic screening is typically only beneficial for more severe disorders. In the case of asthenozospermia, genetic examination usually focuses on absolute asthenospermia. Asthenozospermia can be divided into two categories: isolated, without associated symptoms, and with infectious and inflammatory symptoms of the nose, sinuses, and bronchi. Both categories have the potential to be inherited and are considered genetic diseases[17,18].

The isolated type of disease, known as multiple morphological abnormalities of the sperm flagella (MMAF), is diagnosed by infertility and complete immotile sperm. It is a result of a genetic disorder that affects genes related to the development and function of the sperm tail. The syndromic type of the disease affects genes in the flagella structure of the whole body, including the respiratory system and sperm tail. These diseases include Young and Kartagener syndromes, which involve sinus and lung issues along with infertility. Both diseases are inherited in an autosomal recessive manner, and the likelihood of affected children is very rare if the spouse is not a carrier. Consanguineous marriage should be avoided in both categories, although this is not possible in MMAF due to the lack of clinical manifestations. The difference between the two categories is that in cilia disorders, the tail shape is normal but non-functional, while in MMAF, the tail shape is completely abnormal [17-19].

Asthenospermia can be caused by genetic disorders other than these two diseases, and it is unclear what level of asthenospermia should be genetically examined. Most changes observed in these patients are microdeletions and inversions, with a small percentage showing abnormalities in the karyotype. There is a lack of information in this group due to the small number of patients with isolated asthenospermia, as most severe disorders affect all three categories of count, motility, and morphology. Some physicians request karyotype in these cases, while others postpone it until repeated failure in microinjec-

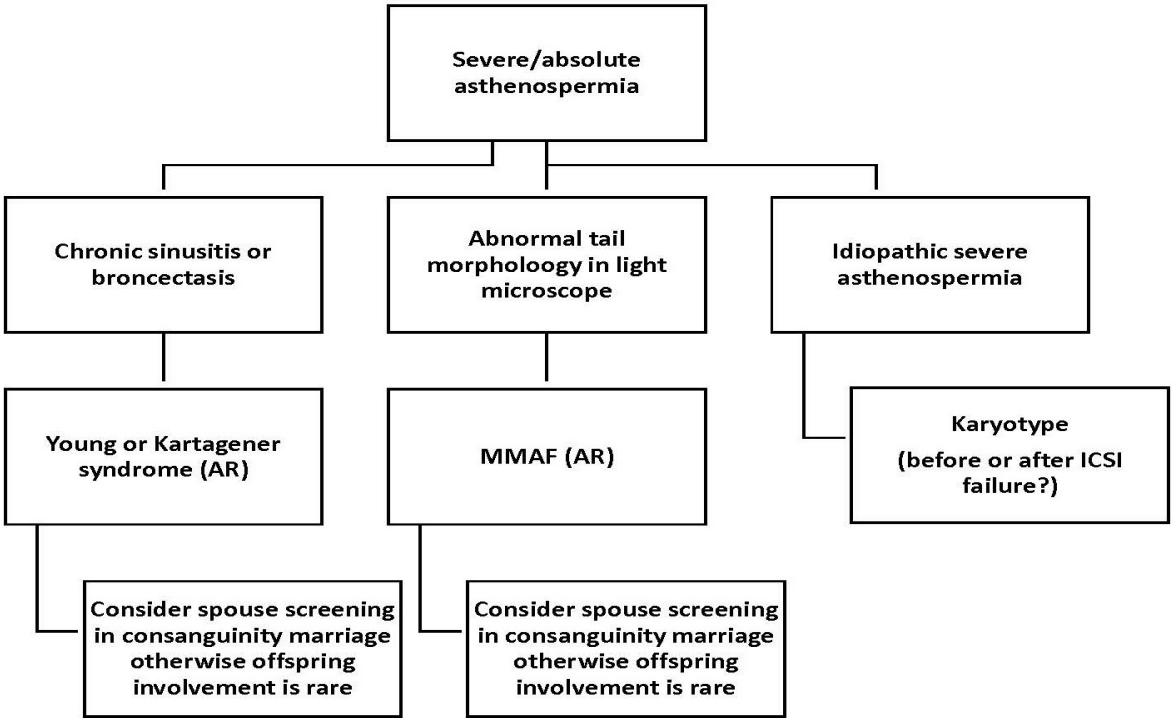

Figure 2. Genetic evaluation in severe asthenospermia

tion. **Figure 2** provides suggestions for genetic testing in asthenospermia[19,20].

Genetic evaluation of absolute teratospermia

Absolute teratospermia, which refers to abnormal sperm morphology, is more controversial and operator-dependent compared to motility and count. Genetic examination is typically only necessary for absolute teratospermia. The three parts of the sperm (head, neck, and tail) are important for morphology, with the sperm tail being particularly relevant in MMAF. The literature discusses absolute head and neck teratospermia. The morphology of the sperm head depends on the acrosome, genetic content of the nucleus, and the ratio of protamine and histone in the nucleus. Variants such as Globozoospermia, macrocephalic sperm, and acephalic sperm have been studied extensively.

Mutations causing these three disorders have been identified and cannot be detected through karyotype analysis due to their small size. Screening methods are available for the most common mutations associated with Globozoospermia, macrozoospermia, and cephalic sperm. These mutations only affect the shape of the sperm and do not have generalized effects on other body systems. Therefore, genetic testing is often skipped before microinjection, although the chance of failure may be higher. The exception is macrozoospermia, which has a high risk of aneuploidy when accompanied by an AURKC mutation.

Genetic evaluation of recurrent abortion and recurrent IVF failure

In terms of recurrent abortion and recurrent IVF failure, there is ongoing debate about whether there are differences in male evaluation between the two conditions. Current literature suggests that there is no established variation in male evaluation for these conditions. However, investigations primarily focus on assessing sperm qualities and genetic contents in both cases. Recurrent miscarriage refers to the loss of fetuses before 20 weeks of fetal age occurring three or more times in a row. Repeated failure of IVF refers to three or more failures in either the embryo formation stage or the implantation stage, without any chemical pregnancy occurring. The probability of genetic causes is much higher in miscarriages before 12 weeks compared to the following eight weeks.

Genetic investigation for these disorders involves examining the genetics of the person through peripheral blood karyotype and examining the genetics of the sperm through sperm karyotype. Reciprocal translocation and Robertsonian translocation are the most common disorders found in the peripheral blood karyotype, accounting for about 3-5% of the causes of abortion in males. Both structural and numerical changes can be found in the sperm karyotype at a similar frequency. The FISH method is a suitable and highly accurate method for genetic examination of sperm.

CONCLUSIONS

In conclusion, there are appropriate genetic approaches for severe abnormalities in semen analysis. Neglecting these approaches can lead to risks for offspring abnormalities or expenses from multiple failed ART cycles. However, using these approaches without following indicated criteria can result in many patients with normal genetic tests.

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