

# Genetics of human male infertility

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## ABSTRACT

Infertility is defined as a failure to conceive in a couple trying to reproduce for a period of two years without conception. Approximately 15 percent of couples are infertile, and among these couples, male factor infertility accounts for approximately 50 percent of causes. Male infertility is a multifactorial syndrome encompassing a wide variety of disorders. In more than half of infertile men, the cause of their infertility is unknown (idiopathic) and could be congenital or acquired. Infertility in men can be diagnosed initially by semen analysis. Seminograms of infertile men may reveal many abnormal conditions, which include azoospermia, oligozoospermia, teratozoospermia, asthenozoospermia, necrospermia and pyospermia. The current estimate is that about 30 percent of men seeking help at the infertility clinic are found to have oligozoospermia or azoospermia of unknown aetiology. Therefore, there is a need to find the cause of infertility. The causes are known in less than half of these cases, out of which genetic or inherited disease and specific abnormalities in the Y chromosome are major factors. About 10–20 percent of males presenting without sperm in the ejaculate carry a deletion of the Y chromosome. This deleted region includes the Azoospermia Factor (AZF) locus, located in the Yq11, which is divided into four recurrently deleted non-overlapping subregions designated as AZFa, AZFb, AZFc and AZFd. Each of these regions may be associated with a particular testicular histology, and several candidate genes have been found within these regions. The Deleted in Azoospermia (DAZ) gene family is reported to be the most frequently deleted AZF candidate gene and is located in the AZFc region. Recently, a partial, novel Y chromosome 1.6-Mb deletion, designated “gr/gr” deletion, has been described specifically in infertile men with varying degrees of spermatogenic failure. The DAZ gene has an autosomal homologue, DAZL (DAZ-Like), on the short arm of the chromosome 3 (3p24) and it is possible that a defective autosomal DAZL may be responsible for the spermatogenic defect. The genetic complexity of the AZF locus on the long arm of the Y chromosome could be revealed only

with the development of sequence tagged sites. Random attacks on the naked mitochondrial DNA (mtDNA) of sperm by reactive oxygen species or free radicals will inevitably cause oxidative damage or mutation to the mitochondrial genome with pathological consequences and lead to infertility in males. The key nuclear enzyme involved in the elongation and repair of mtDNA strands is DNA polymerase gamma, mapped to the long arm of chromosome 15 (15q25), and includes a CAG repeat region. Its mutation affects the adenosine triphosphate production. The introduction of molecular techniques has provided great insight into the genetics of infertility. Yet, our understanding of the genetic causes of male infertility remains limited.

**Keywords:** genetic counselling, infertility, oligozoospermia, semen analysis, Y chromosome

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## INTRODUCTION

One would ordinarily imagine that in a country like India, which already has a population of over 980 million people, child bearing should be an effortless endeavour. This, however, is not true. Quite unrelated to the national population figure is the prevalence of infertility. Male factor infertility accounts for approximately 50% of causes for infertile couples, who make up 15% of all couples. Researchers have shown great interest in correctly estimating a man's fertility potential. The term “male infertility” does not constitute a defined clinical syndrome, but rather, a collection of different conditions exhibiting a variety of aetiologies and a varying diagnosis. The World Health Organisation (WHO) has defined infertility as a period of two years without conception, but many couples actually seek a medical opinion after one year of infertility. In men, oligozoospermia, asthenozoospermia, teratozoospermia and azoospermia are the main causes of infertility, and these account for 20%–25% of cases. Because modern artificial reproduction techniques like intracytoplasmic sperm injection (ICSI) can help couples to overcome infertility, it is imperative to analyse the underlying genetic causes of male infertility. In this article, we present a brief overview of the genetic complexity of the human Y chromosome, autosomal genetic factors, mitochondrial mutations and genetic counselling in the context of human male infertility.

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## INCIDENCE AND PREVALENCE OF MALE INFERTILITY

It is estimated that globally, 60–80 million couples suffer from infertility every year, of which probably 15–20 million are in India alone. This is an equally important national problem concerning reproductive health. The magnitude of the problem calls for urgent action, particularly when the infertility is avoidable in the majority of cases.

## AETIOLOGY OF MALE INFERTILITY

Infertility is either primary, when no pregnancy has ever occurred, or secondary, where there has been a pregnancy, regardless of the outcome. Primary and secondary infertility is found in 67%–71% and 29%–33% of patients, respectively. About one in ten couples are infertile for several possible reasons, with the male factor being responsible for approximately 50% of the cases. This group is typically split into 30% with strictly male factors, and 20% with both male and female factors. However, in approximately 30% of cases, the origins of reduced male infertility are unknown. Male infertility is a multifactorial syndrome encompassing a wide variety of disorders. In more than half of infertile men, the cause of their infertility is unknown (idiopathic) and could be congenital or acquired. Up to 10% of infertility cannot be explained medically. Some of the male infertility disorders are summarised in Fig. 1.

The known causes of male infertility are quite numerous but can be grouped into a number of major categories. Male infertility has been associated with several genetic and non-genetic conditions.

### Genetic or molecular causes of male infertility

Genetic abnormalities have been identified in men with unexplained oligozoospermia and azoospermia, including numerical and structural chromosomal abnormalities.<sup>(2)</sup> Genetic factors involved in male infertility manifest as chromosomal disorders, mitochondrial DNA (mtDNA) mutations, monogenic disorders, multifactorial disorders and endocrine disorders of genetic origin.

### Chromosomal disorders

Human male infertility is often related to chromosome abnormalities. Klinefelter syndrome (XXY) and specific translocations are well-established causes of male infertility.<sup>(3)</sup> Two important gene defects conclusively associated with spermatogenic failure are the point mutations in the androgen receptor and the cystic fibrosis transmembrane conductance regulator (CFTR) gene commonly associated with congenital vas deferens abnormalities.<sup>(4)</sup> The most frequent sperm chromosome

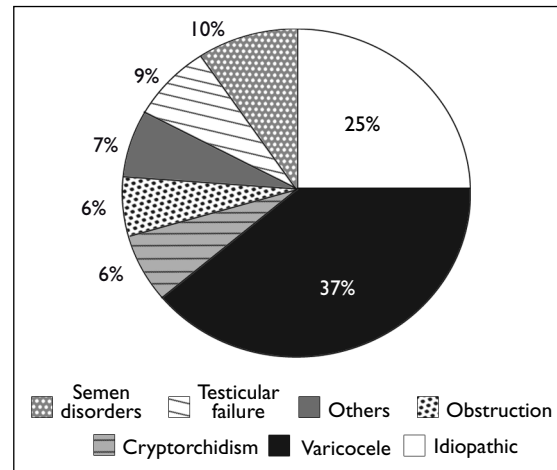


Fig. 1 Pie chart shows the distribution of male infertility disorders.<sup>(1)</sup>

anomaly in infertile males is diploidy, originated from either meiotic mutations or by a compromised testicular environment.

### (a) Microdeletion of the Y chromosome

One of the most significant pathogenetic defects associated with male infertility is microdeletions of the long arm of the Y chromosome (Yq). 13% of azoospermic men, 1%–7% of severely oligozoospermia men, 5% of men with severe primary testicular failure and with a sperm density of less than 5 million/ml showed Y chromosome microdeletion.<sup>(3)</sup> De novo deletions of Yq are one of the most frequently-occurring chromosomal abnormalities in men and are believed to arise from recombination events between long stretches of highly repetitive DNA sequences during meiosis or early pre-implantation development.<sup>(5)</sup> Accordingly, Y chromosome microdeletions contribute only marginally to the totality of human male infertility, but when present, the introduction of ICSI as an artificial reproduction technique may allow for the transmission of such mutations to the next generation.<sup>(6)</sup>

### (b) Mitochondrial DNA mutations

Apart from some nuclear genes, mitochondria have their own genome, capable of producing many essential components of the respiratory chain that have a profound impact on sperm motility. The quality and quantity of sperm production may be affected greatly by both environmental and genetic factors. Sperm mitochondria play an important role in spermatozoa because of the high adenosine triphosphate (ATP) demand of these cells.<sup>(7)</sup>

### (c) Monogenic disorders

Monogenic genetic disorders occur as a direct consequence of a single gene being defective. Such disorders are inherited in a simple pattern according to Mendel's Laws.

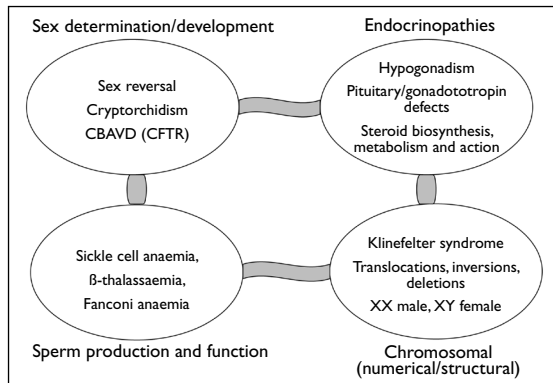


Fig. 2 Chart shows the genetic aetiologies of male infertility<sup>(9)</sup>

There are over 50 monogenic disorders associated with male infertility, in a few of which the primary genetic defect has been described.

#### (d) Multifactorial disorders

Multifactorial disorders result from mutations in multiple genes, often coupled with environmental causes. A mutation (C677T) in the gene, methylenetetrahydrofolate reductase (MTHFR), is known to increase susceptibility to various multifactorial disorders.<sup>(8)</sup> A few important genetic causes associated with male infertility are shown in Fig. 2.

#### Significance of Y chromosome microdeletions in male infertility

Some abnormalities associated with infertility are inherited, like reciprocal and Robertsonian translocations and CFTR mutations. Of all the genetic factors, the study of Y chromosome microdeletions is particularly important because of the potential for transmission of genetic abnormalities to the offspring, as these techniques bypass the physiological mechanisms related to fertilisation. Quite recently, sperm mitochondrial mutations have been gaining much attention.

#### **Non-genetic causes of male infertility**

Non-genetic causes include hypogonadotropic hypogonadism, testicular maldescence, structural abnormalities of the male genital tract (obstruction of spermatic ducts, agglutination of sperm), genital infections, impotency, previous scrotal or inguinal surgery, varicoceles, chronic illness, medication, exposure to chemicals, environmental factors and immunological causes.

#### (a) Hormonal causes

The study of endocrine disruptors has raised concerns about the reproductive effects of exposure to certain environmental compounds that affect the endocrine system via oestrogenic, androgenic, anti-androgenic

and antithyroid mechanisms. Elevated concentrations of luteinising hormone (LH) and follicle-stimulating hormone and low concentrations of gonadal steroids cause gonadal failure, a reason for infertility. In the male, elevated concentrations of LH can result from hypergonadotrophic hypogonadism (HH) which could be due to various causes, such as primary testicular failure, seminiferous tubule dysgenesis (Klinefelter syndrome), Sertoli cell failure and anorchia.<sup>(10)</sup>

#### (b) Hypogonadotrophic hypogonadism

Patients with HH show decreased levels of gonadotrophins.

#### (c) Impotency

Impotence or erectile dysfunction affects 10%–15% of all males and can be emotionally and psychologically disabling for men and their partners. Physical factors such as drugs, blood flow abnormalities, nerve impulse abnormalities and hormonal abnormalities are the major causes in 80% of erectile dysfunction cases. Psychological factors account for the remaining cases and may be attributed to stress, performance anxiety or misinformation about sexuality.

#### (d) Previous scrotal or inguinal surgery

Gonorrhoea and chlamydia cause acute inflammation of the scrotal contents (usually unilateral) in young men. Painless swellings in the scrotum are common. Most of these are small, round, epididymal cysts or spermatoceles that require no investigation or treatment.<sup>(11)</sup>

#### (e) Varicocele

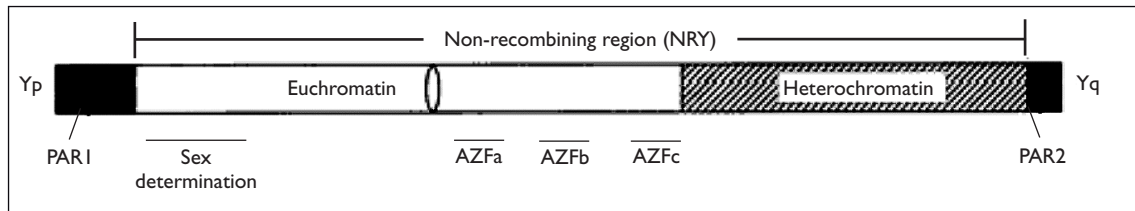
The most common identifiable cause of male subfertility is a varicocele, a condition of palpably distended veins of the pampiniform plexus of the spermatic cord.<sup>(12)</sup> Varicoceles feel like a bag of worms in the scrotum and can be associated with infertility.<sup>(11)</sup> The term “subclinical varicocele” refers to a lesion too small to be detected by physical examination.

#### (f) Exposure to chemicals

Various chemicals have been implicated as reproductive toxicants. Air pollutants are present in the blood, urine and semen of exposed men and may affect sperm quality.<sup>(13)</sup> Sperm function tests have shown that high lead levels in semen samples reduce the ability of the sperm to bind to the egg, and also to penetrate and fertilise the egg.

#### (g) Environmental factors

Controversy exists over decreasing human sperm concentrations worldwide. Occupational heat exposure is a significant risk factor for male infertility, affecting



**Fig. 3** Diagram shows the schematic structure of the Y chromosome.<sup>(17)</sup>

sperm morphology and resulting in delayed conception. The male reproductive system is particularly vulnerable to the effects of the chemical and physical environment. This may be due to dramatic events or to endemic conditions of the environment. In this respect, industrial and agricultural pollution are of significant concern. Idiopathic male infertility may be due to exposure to environmental toxicants that alter the reproductive hormones, spermatogenesis or sperm function. Semen quality has been shown to be altered on exposure to environmental ozone.<sup>(14)</sup> Environmental reproductive hazard studies report that sperm counts have declined in certain industrialised countries.

#### (h) Immunological factors

Because immunological factors operate at almost every step in the human reproductive process, antibodies-induced damage to gametes is a major cause of immunological infertility. Lifestyle and environmental factors,<sup>(15)</sup> including smoking, can affect gamete, leading to subfertility/infertility.

### Y CHROMOSOME: THE GENETIC ESSENCE OF MALE INFERTILITY

The Y chromosome is one of the smallest human chromosomes and consists of a short (Yp) and a long (Yq) arm. The Y chromosome is male-specific, 60 megabases (Mb) in size, consisting of 60 million nucleotides, but has the least number of genes compared to any other chromosome. Of the 27 Y chromosome genes identified, nine are located on the Yp and the remaining 18 on the Yq. In 2003, Skaletsky et al reported the complete sequence of the 23 Mb euchromatin segment, in which they designated the MSY (for male specific region of the Y).<sup>(16)</sup> 95% of the Y chromosome is covered by MSY, which represents a mosaic of three classes of euchromatic (X-transposed, X-degenerate and ampliconic) and heterochromatic sequences. Thus far, 156 transcription units, 78 protein-coding genes and 27 distinct proteins of the Y chromosome have been identified. A schematic representation of the Y chromosome is provided in Fig. 3.

The pseudoautosomal regions (PARs), which pair with the X chromosome during meiosis, are located at both

ends of the Y chromosome. The region outside the PARs that does not recombine is called the non-recombining region of the Y chromosome (NRY). The Yp and the proximal part of the Yq consist of euchromatin, while the distal part of the Yq is made up of heterochromatin, and this region may vary in length to constitute about one-half to two-thirds of the Yq. Therefore, the Yq may be cytogenetically divided into an euchromatic proximal region (Yq11, subdivided into Yq11.1, 11.21, 11.22, and 11.23) and a heterochromatic distal region (Yq12), whereas the euchromatic short arm is called the Yp11.<sup>(17)</sup> Recently, molecular methods have identified the loci involved in the production and differentiation of the sperm. The Y chromosome has been divided into seven deletion intervals. Each of these intervals is further subdivided into subintervals (A, B, C, etc.). In 1992, Vollrath et al constructed a 43-interval deletion map of the human Y chromosome that contained an ordered array of sequence tagged sites (STS) which span the entire length of the Y chromosome.<sup>(18)</sup> The short arm and the centromere contain intervals 1–4, distal to proximal; the euchromatic part of the Yq is represented by intervals 5 and 6, proximal to distal; the heterochromatic region is defined as interval 7. Deletion interval 5 corresponds approximately to Yq11.21 through the middle part of Yq11.22, and deletion interval 6 corresponds to the middle part of Yq11.22–Yq11.23.

#### Human Y chromosome deletion mapping

In 1976, Tiepolo and Zuffardi were the first to hypothesise a correlation between Y chromosome deletions and male infertility.<sup>(19)</sup> Yq microdeletions were detected in 5%–15% of males with spermatogenic failure. These deletions include the total Yq12 heterochromatin block and at least part of the adjacent euchromatin part of the Yq (Yq11.23), and are clustered within intervals 5 and 6 of the Y chromosome. Consequently, it was postulated that at least one genetic Y factor essential for male germ cell development is located in the distal Yq11. It was defined as the Y borne fertile gene or Azoospermia Factor (AZF). The AZF, found on the Yq, may be the most thoroughly studied pure male sterile locus in humans. It has been suggested that the AZF resides in the distal non-fluorescent region on the Yq.

### Three different AZF regions exist in Yq11

The AZF region is further subdivided into three non-overlapping hot spot regions defined as AZFa, AZFb and AZFc.<sup>(20)</sup> Furthermore, recently a fourth region, AZFd, has been proposed between AZFb and AZFc. So far at least 12 genes have been isolated from these regions.<sup>(21)</sup> Several genes located in the AZF regions are expressed in the testes and could therefore be viewed as “AZF candidate genes”. Fig. 4 depicts the three different AZF regions of the Y chromosome.

#### The AZFa region

The AZFa is located at the proximal portion of deletion interval 5 (subinterval 5C) and it has been estimated to span 400–600 kb of DNA.

##### (a) Candidate gene

DFFRY (Drosophila fat facets-related Y), recently renamed as USP9Y (ubiquitin-specific protease 9, Y chromosome), was the first functional gene identified in the AZFa and subsequently shown to be absent in infertile patients.<sup>(22)</sup> This gene, USP9Y, occupies less than half of the AZFa interval, while the majority of infertile males carrying AZFa deletions display the absence of this entire interval.<sup>(20)</sup> The most recent data suggests that the AZFa phenotype requires more than one gene. They include DBY (DEAD box on the Y) and UTY (ubiquitous TPR motif on the Y). DBY consists of 17 exons,<sup>(23)</sup> and encodes for a putative ATP-dependent RNA helicase, as it belongs to the DEAD-box proteins.<sup>(24)</sup> However, its specific function in male germ cell development is still unknown. These findings suggest that other gene(s) in this region may be responsible, either singly or in combination with USP9Y, for the spermatogenic disruption observed in AZFa-deleted patients.

##### (b) Genotype/phenotype correlation

In the majority of cases involving the AZFa interval, a Sertoli cell-only (SCO) syndrome phenotype is observed;<sup>(20)</sup> either no germ cells are visible in any seminiferous tubules (SCO I) or germ cells are present in a minority of tubules. The latter variant arises from a failure to complete differentiation and maturation of spermatocytes and spermatids, leading to the degeneration of germ cells within most tubules (SCO II). The testicular histology of AZFa patients with azoospermia always show SCD, while in patients with severe oligozoospermia, it resembles severe hypospermatogenesis, i.e. no maturation arrest is seen.

#### The AZFb region

The AZFb spans from the distal portion of deletion interval 5 to the proximal end of deletion interval 6 (subinterval 5O–6B) and it spans around 1–3 Mb of DNA.

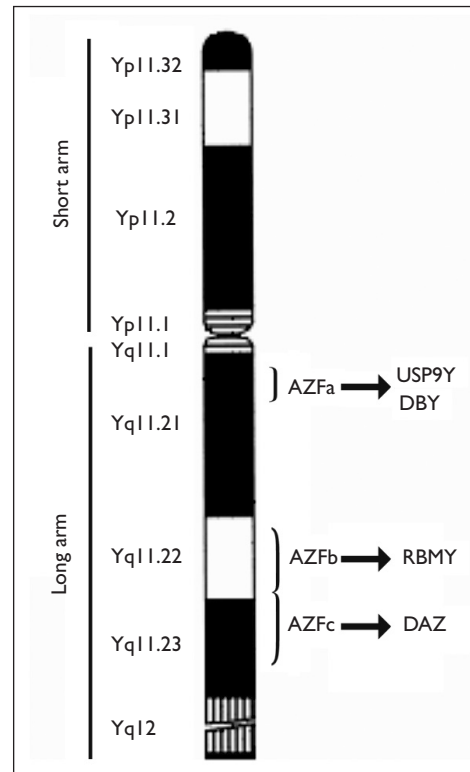


Fig. 4 Diagram shows the AZF region of the Y chromosome.<sup>(17)</sup>

##### (a) Candidate gene

So far, two genes EIF1AY (translation-initiation factor 1A, Y isoform) and RBMY (RNA binding motif on the Y) have been mapped in the AZFb region. EIF1AY encodes a Y isoform of eIF-1A, an essential translation initiation factor. EIF1AY is not considered as an AZFb-candidate gene since its role in spermatogenesis is completely unknown, and no deletion specifically removing this gene has been reported. RBMY was the first among the AZFb candidate genes to be identified. There is a family of 20–50 genes and pseudogenes spread across both arms of the Y chromosome, including a cluster within the AZFb region,<sup>(25,26)</sup> and YRRM has been renamed as the “RBMY gene family”. The RBMY proteins at the N terminus contain a single RNA-binding domain of the RRM (RNA recognition motif) type and an auxiliary C-terminal domain containing four 37-amino acid (aa) repeats. This domain is known as the SRGY box, since it contains a serine-arginine-glycine-tyrosine sequence.

##### (b) Genotype/phenotype correlation

AZFb patients could have more variable defects, and in about half of these cases, a spermatogenic arrest is observed. The variable spermatogenic alterations observed in AZFb patients may indicate multiple functions of RBMY during spermatogenesis, or alternatively, that other genes located in this region may act in combination with RBMY and that their presence or absence modulates the phenotype.<sup>(17)</sup> Patients with large AZFb

**Table I. AZF candidate genes.**

Gene	Length	Frequency	Important candidate gene	Genotype/phenotype
AZFa	792 kb	1%–2%	DFFRY or UPS6Y, DBY	SCO syndrome
AZFb	1–3 Mb (< 3.2 Mb)	Intermediate, between AZFa and AZFc	RBMV	Maturation arrest of germ cells and azoospermia
AZFc	3.5 Mb	2%–10%	DAZ, CDY	Mild/severe oligozoospermia, azoospermia
AZFd		Not determined		Mild oligozoospermia or even normal sperm counts with abnormal sperm morphology

deletions are azoospermic and those harbouring partial AZFb microdeletions present with a range of infertile phenotypes, including mild and severe oligozoospermia.

#### The AZFc region

AZFc is located at the distal part of deletion interval 6 (subintervals 6C–6E) on the Y chromosome. Deletions of the AZFc region are most frequently found, in 2%–10% of azoospermic or severely oligozoospermic men.<sup>(19)</sup> The AZFc region spans 3.5 Mb and contains seven gene families that are all thought to be involved in spermatogenesis.

#### *(a) Candidate gene*

Deleted in azoospermia (DAZ) is the prime candidate gene in the AZFc region. Several genes other than DAZ have been mapped to the AZFc region, including CDY1 (chromodomain Y 1), BPY2 (basic protein Y 2), PRY (PTA-BL related Y) and TTY2 (testis transcript Y 2). The function of these genes is unknown, but they share similar characteristics: there are multiple copies on the Y chromosome, they are expressed in the testes only, and they are Y specific. Two CDY1 genes are mapped in the AZFc region, one within the DAZ cluster and the other at the distal end. This finding is fascinating since at least one CDY1 copy is invariably absent in patients with DAZ deletion. Therefore, CDY1 can be considered to be an AZFc-candidate gene, but deletions removing this gene specifically should be identified in patients to confirm this assumption.<sup>(17)</sup>

#### *(b) Genotype/phenotype correlation*

Deletions of AZFc are associated with a wide range of phenotypical features ranging from azoospermia to mild to severe oligozoospermia. Table I highlights the candidate genes in each of the AZF regions and their associated genotype/phenotype.

#### The AZFd region

The AZFd region is found between AZFb and AZFc. STS markers like SY133, SY145, SY153, and SY152 are used for screening the AZFd region.

#### *(a) Candidate gene*

No candidate gene has been identified till now. However, detection of the deletion of the DYS237 locus of the AZFd region has frequently been identified by Muslumanoglu et al, who indicated the possible importance of the genes located in this region in spermatogenesis.<sup>(27)</sup> Although many variations in deletion length have been reported, the most frequently noted deletion extends from the STS marker, SY153, in AZFd to the junction of the euchromatic and heterochromatic regions.

#### *(b) Genotype/phenotype correlation*

Patients with microdeletions restricted to AZFd may present with mild oligozoospermia or even a normal sperm count associated with abnormal sperm morphology.

### **DAZ AS A CANDIDATE GENE**

Among cases with Yq microdeletions, deletions involving the DAZ gene family make up the most frequent findings. Mutations in DAZ are associated with 13% of cases of human male infertility. 10%–15% of azoospermic men have shown complete deletion of the DAZ gene. DAZ was acquired by the Y chromosome from an autosomal homologue, DAZ-Like (DAZL), located on chromosome 3p24 and with a single DAZ repeat. The size of the DAZ gene cluster is about 380 kb, consisting of seven copies of DAZ, of which four copies are located relatively close together in deletion interval 6 on the Y chromosome (Yq11). These four copies of DAZ genes exist in two clusters, each comprising an inverted pair (3'← 5': 5'→ 3' orientation) of DAZ genes; cluster 1 consists of DAZ1 and DAZ2, cluster 2 consists of DAZ3 and DAZ4. All four DAZ genes have identical (> 99.9%) DNA sequences. However, they are distinguished by single nucleotide polymorphisms in three STS markers, namely sY581, sY586 and sY587.<sup>(28)</sup> Although most deletions involve a deletion of all four DAZ genes, an absence of only two of the DAZ genes is also associated with impaired spermatogenesis.

The single nucleotide variants that were used to distinguish between the DAZ genes were all located in introns. The coding regions of all four genes appeared

to be intact, with no evidence of frameshift or nonsense mutations in DAZ1, DAZ2, DAZ3 or DAZ4. Indeed, only one coding sequence difference among the DAZ genes was observed: a silent C to T transition in exon 7a in DAZ2. The four DAZ genes differ in size: the approximate sizes of the genes are as follows: DAZ1, 65kb; DAZ2, 70kb; DAZ3, 50kb; DAZ4, 55kb.<sup>(28)</sup> In central positions of all the four genes, there are tandem arrays of a 2.4-kb unit and it contains a 72-bp exon (exon 7) encoding a 24-amino acid segment that is tandemly amplified within predicted DAZ proteins.<sup>(21)</sup> Although frequent deletion of the DAZ genes suggests that DAZ plays an important role in spermatogenesis, the variable penetration of AZFc deletions that remove the entire DAZ gene cluster is also consistent with a certain degree of functional redundancy.

#### **AUTOSOMAL DAZL AS A CANDIDATE GENE**

The DAZ gene has an autosomal homologue, DAZL, on the short arm of the chromosome 3 (3p24). It is highly homologous to the DAZ gene, with 83% similarity in the coding region of the cDNA. Both DAZ and DAZL are the RNA binding proteins.<sup>(29)</sup> DAZL, in turn, is believed to be the father of the DAZ gene, which arose 30–40 million years ago. In 1994, Lilford et al suggested that up to 60% of undiagnosed male infertility arises from autosomal recessive mutations.<sup>(30)</sup> A novel mutation, i.e. A G transition at nucleotide 386 in exon 3 of the DAZL gene, was identified in some infertile patients. The mutation is located within the RNA-recognition motif (aa 32–117) domain of the DAZL protein and will lead to Thr54 Ala change (T54A) of the DAZL protein.<sup>(31)</sup>

There are, however, no reported instances of DAZL gene mutations among infertile men in India.<sup>(32)</sup> Whether DAZL plays a crucial role in spermatogenesis in humans also merits investigation. Given this evidence of the functional redundancy for DAZ, it would be tempting to postulate that DAZL and DAZ operate in a harmonising or synergistic manner during human spermatogenesis. If proven, DAZL seems to be an attractive candidate for autosomal recessive infertility.

#### **gr/gr DELETIONS**

The classical AZFc deletion, which removes 3.5 Mb between the b2/b4 amplicons, is the most common type of deletion. In view of the Y chromosome structure and the suggested deletion mechanism, a number of other possible partial deletions have been proposed in both the AZFb and AZFc regions.<sup>(33)</sup> The frequency and pathological significance of these partial deletions is not yet clear, although recently, a partial, novel, Y chromosome 1.6-Mb deletion, designated the “gr/gr” deletion, has been

described specifically in infertile men with varying degrees of spermatogenic failure.<sup>(34)</sup>

The gr/gr deletion removes part of the AZFc region, including two copies of DAZ and one copy of CDY1 as well as several other transcription units. Since father-to-son transmission is observed, the gr/gr deletion likely results in subfertility rather than complete infertility. The deletion has been observed to be in association with numerous Y haplotypes, which suggests multiple independent recombination events. Another deletion, named b2/b3 or u3-gr/gr or g1/g3, which removes a similar quantity of AZFc genes, has also been identified.

#### **DNA POLYMERASE GAMMA**

Sperm mitochondria play an important role in spermatozoa functionality; therefore, genetic alterations to mtDNA may have consequences for normal fertilisation. The key nuclear enzyme involved in the elongation and repair of mtDNA strands is DNA polymerase gamma (POLG), believed to be the only polymerase acting in the mitochondria. The catalytic subunit of POLG is encoded by the POLG gene, which has been mapped to Yq 15 (15q25) and includes a CAG repeat region.<sup>(35)</sup> Mutations in the POLG gene affect the proofreading activity of the enzyme, which leads to mutation in the mitochondrial genome and subsequently affects the ATP production.

Exon 1 of the human POLG contains a CAG trinucleotide repeat, which codes for polyglutamate. Expanded CAG repeats in a different locus in the genome have been reported to be mostly associated with neurological disorders. The association of a large stretch of CAG repeat with male infertility is not exceptional. Trinucleotide repeat sequences are associated with at least 11 human genetic diseases including myotonic dystrophy type 1, Huntington’s disease, spinal and bulbar muscular atrophy and Machado-Joseph disease, etc. Expansion of the POLG CAG repeat would be a plausible disease mechanism in disorders characterised by mtDNA instability inherited as an autosomal trait, since expansion of the polyglutamate trait might impair the function of the mtDNA polymerase in a dominant-negative manner. Numerous studies have indicated an association between different polymorphisms, mutations or deletions in the mitochondrial genome and sperm dysfunction.<sup>(36)</sup> One of these studies was particularly informative as it identified specific mtDNA haplogroups that are associated with asthenozoospermia.

#### **MITOCHONDRIAL DNA MUTATIONS AND MALE INFERTILITY**

##### **Mitochondrial DNA**

Mitochondria are the intracellular organelles responsible

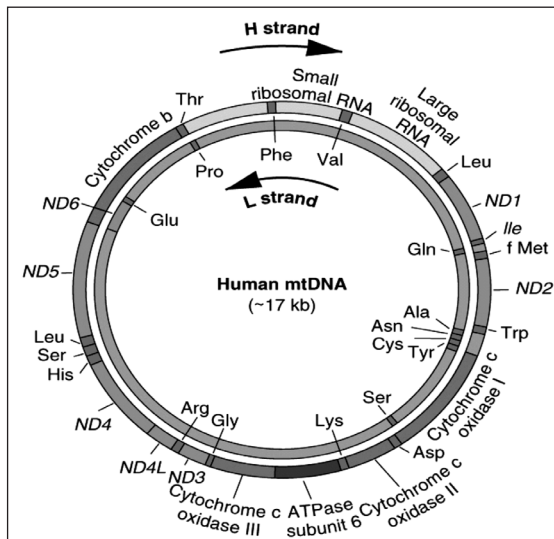


Fig. 5 Diagram shows mitochondrial DNA.<sup>(40)</sup>

for energy metabolism in eukaryotic cells.<sup>(37)</sup> Three decades ago, it was established that human and animal cells contain a second genome in the mitochondria. Human mtDNA is a 16569-bp double-stranded circular DNA molecule that codes for 2rRNAs, 22tRNAs and 13 polypeptides, essential components of four respiratory enzyme complexes.<sup>(38)</sup> mtDNA has no introns and both strands are transcribed to synthesise a functional protein synthesis machinery in the mitochondria. mtDNA mutates at a rate 10–20 times higher than nuclear DNA.<sup>(39)</sup> This high mutation rate is due to the peculiar structure and unique replication system of mtDNA. The structure of human mtDNA is shown in Fig. 5.

#### Mitochondrial DNA mutations in human sperm

Spermatozoa need a great deal of energy to support their rapid movement after ejaculation. Thus, random attacks on the naked mtDNA of sperms by reactive oxygen species (ROS) or free radicals will inevitably cause oxidative damage or mutation to the mitochondrial genome with pathological consequences, and lead to infertility in males.<sup>(37)</sup> The midpiece of the mammalian sperm contains 70–80 mitochondria and there is one copy of mtDNA in each mitochondrion.

About 85% of sperm samples contain large-scale mtDNA deletions of variable sizes and most spermatozoa have 2–7 deletions of mtDNA. It has been pointed out that an age-related increase in oxidative stress and oxidative damage to mtDNA is involved in male infertility. Among the mitochondrial deletions observed, the so-called “common deletion” of 4977 bp is the most prevalent and abundant. It remains to be studied whether the mtDNA deletions are localised in the same mitochondria or in different organelles of spermatozoa harbouring mutated mtDNA molecules. Some novel point mutations of

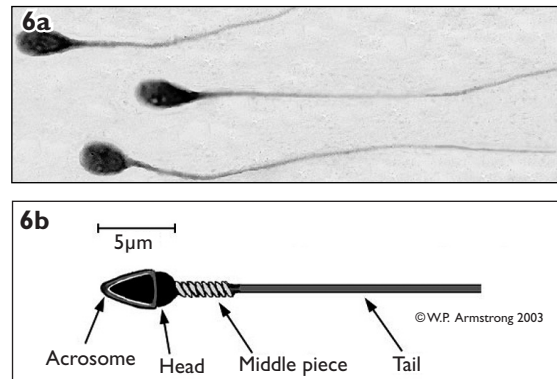


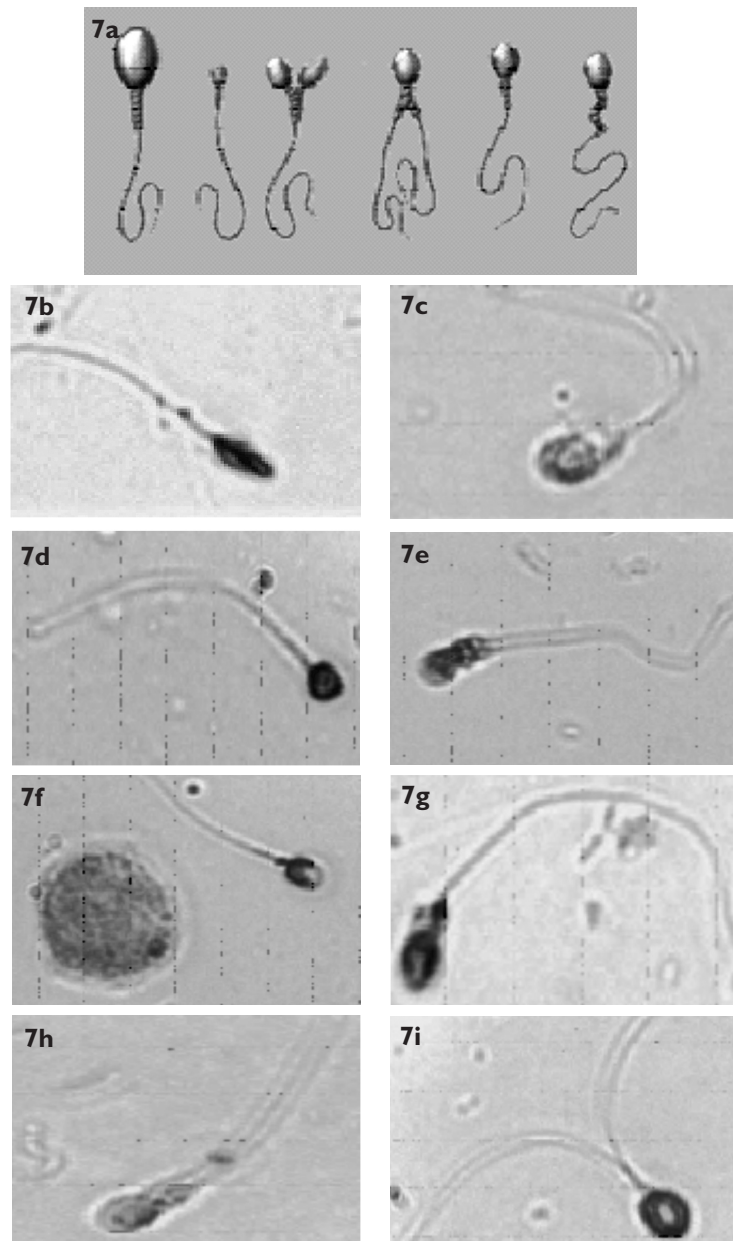
Fig. 6 (a) Photomicrograph of human spermatozoa in semen ( $\times 1,000$ ), and (b) illustration of a normal sperm morphology.<sup>(45)</sup>

mtDNA are present in some of the spermatozoa with poor motility or in spermatozoa of infertile males. Thangaraj et al in 2003 also observed a novel 2-bp deletion (nucleotides 8195 and 8196) in the COII gene, which might have given rise to a truncated protein.<sup>(41)</sup> Holyoake et al in 2001 found the two most common substitutions at 9055 and 11719 in men, with a significantly higher frequency of reduced sperm motility.<sup>(42)</sup>

The mtDNA mutations detected so far may just represent the “tip of the iceberg” of all possible mutations in spermatozoa. Since sperms require a substantial amount of energy to swim fast enough to reach the oviduct during fertilisation, the appropriate bioenergetic function of mitochondria is critical in order to avoid male infertility.<sup>(36)</sup> There is an increasing amount of evidence that mtDNA anomalies in sperms may lead to infertility. Poor sperm quality may be due to point mutations, deletions and the presence of a specific mtDNA haplogroup. Very low levels of somatic mtDNA deletions have been identified in the semen of infertile men. It has been suggested that these mutations cause infertility through an effect on sperm motility.

Ejaculated spermatozoa, particularly in infertile men, have been shown to display numerous features that are typical of apoptosis in somatic cells, including Fas expression, ROS production, activation of caspases, DNA fragmentation, reduction in mitochondrial membrane potential, plasma membrane translocation of phosphatidylserine and permeability. It has been shown that high levels of A3243G mtDNA mutant strongly correlate with low sperm motility. The important role of genetic abnormalities in the causation of human male infertility is increasingly recognised. While much remains to be learnt in this fast-moving field, considerable progress has been made in the clinical delineation of genetic forms of male infertility and in the characterisation of the responsible genes and their mutations or deletions.





**Fig. 7** (a) Illustration shows the various abnormal sperm structures.<sup>(46)</sup> Photomicrographs show the following types of abnormal sperm morphologies: (b) tapering head; (c) cytoplasmic droplets; (d) pinhead; (e) pyriform head with defective midpiece; (f) pyriform head with blood cell; (g) small oval head with deformed midpiece; (h) cytoplasmic droplet attached to a normal head; and (i) double tailed.

### EVALUATION OF AETIOLOGY

Previously, infertility in men could only be diagnosed by semen analysis. Thanks to the application of modern molecular techniques, there is reason to hope that knowledge of male subfertility is moving from an era in which it was defined by the properties of semen to one in which it is seen as the result of one of several identifiable genetic or pathological mechanisms. It has become clear that much male infertility is genetic. However, the causes of most types of male infertility remain unclear.

### Semen analysis

A high quality basic semen analysis is one cornerstone

in the investigation of the infertile couple. This test is inexpensive, easy to perform and provides valuable information. Semen analysis comprises a set of descriptive measurements of spermatozoa and seminal fluid parameters that help to estimate semen quality. Conventional semen analysis includes measurement of particular aspects of spermatozoa such as concentration, motility and morphology, and of seminal plasma. The concentrations of motile spermatozoa and/or quality of sperm motility are the most important semen parameters related to conception. To date, semen analysis is only one indicator of the ability to conceive a child. As per WHO norms, a normal male should have more than

**Table II. WHO normal ranges for semen analysis.<sup>(43,44)</sup>**

Semen analysis	WHO (1987)	WHO (1992)
<b>Semen characteristics</b>		
Volume (ml)	≥ 2	≥ 2
pH	7.2–8.0	7.2–8.0
Sperm concentration (M/ml)	≥ 20	≥ 20
Total sperm count (M/ejaculate)	≥ 40	≥ 40
Morphology (% normal)	≥ 50	≥ 30
Vitality (% live)	≥ 75	≥ 75
WBC (M/ml)	< 1.0	< 1.0
Immunobead test (% sperm with beads)	≥ 10	< 20
MAR test (% sperm with RBCs)	< 10	< 10
<b>Motility within 1 h of ejaculation</b>		
Class a (%)	≥ 25	≥ 25
Classes a and b (%)	≥ 50	≥ 50
Neutral alpha-glucosidase (mU/ejaculate)		≥ 20
Total zinc (µmol/ejaculate)		≥ 2.4
Total citric acid (µmol/ejaculate)		≥ 52
Total acid phosphatase (U/ejaculate)		≥ 200
Total fructose (µmol/ejaculate)		≥ 13

20 million sperms/ml of semen. The normal range for various semen characteristics is depicted in Table II. Normal and abnormal sperm morphologies are shown in Figs. 6 and 7.

Sperm abnormalities (Table III) are categorised by whether they affect sperm count, sperm quality (motility and morphology), or both. Measures of semen quality are used as a surrogate measure of male fecundity in clinical andrology. The implications of even moderate alterations in semen quality are poorly understood and only limited data is available for relating these measures to the likelihood of achieving pregnancy.

### STS mapping of the Y chromosome

The genetic complexity of the AZF locus located in the Yq could be revealed only with the development of STS. These analyses permit the detection of interstitial submicroscopic deletions not visible at the cytogenetic level and detectable only by STS-PCR or southern hybridisation. Such deletions are called microdeletions.<sup>(17)</sup> Since the first cytogenetic report of Y-deleted segments in 1976, mapping studies on the Y chromosome has been greatly facilitated by the use of polymerase chain reaction (PCR) techniques to amplify known markers (STSs) spanning the deleted regions.

As a direct result of the human genome project, more than 200 such markers are now known and they span the entire Y chromosome. Over 15 studies have demonstrated that a number of these markers are absent in 10%–30% of infertile men, indicating that their Y chromosomes have certain deleted segments. Generally, fertile controls do

**Table III. WHO nomenclature for semen variables.<sup>(47)</sup>**

Medical name	Description
Azoospermia	Complete absence of sperm.
Aspermia	Ejaculation does not emit any semen.
Oligozoospermia	< 10 million sperm/ml of semen.
Asthenozoospermia	> 40% of sperm have low motility.
Teratozoospermia	> 40% of sperm with abnormal morphology.
Necrospermia	Non-viable / dead sperm.
Oligoasthenozoospermia	Motile density < 8 million sperm/ml.
Hematospermia	Red blood cells present in semen.
Pyospermia	White blood cells present in semen.
Polyzoospermia	Excessively high sperm concentration.

not have these deletions. The missing stretches in the Y chromosomes are submicroscopic and are not large enough to be detected by conventional chromosomal analysis. Deletions most frequently involve the AZFc region including DAZ, less frequently the AZFb region including RBMY, and rarely the AZFa interval. Larger deletions involving two or three AZF regions are also observed, while in some cases the deletions are located in regions not overlapping with the AZF intervals.

### Importance of screening the AZF region

The identification of the actual role played by the AZF candidate genes would significantly advance our understanding of the biology of spermatogenesis. In addition, an analysis of novel Y-chromosomal genes with a potential role in male germ cell development would clarify other important and useful features of this important chromosome.<sup>(17)</sup> Further, it may suggest whether the patient can undergo ICSI.

In most andrology and infertility centres in India and abroad, Y chromosome microdeletion assay is performed for infertile males whose sperm density is below 5 million/ml. This is an essential prerequisite for infertile men undergoing ICSI to rule out the possibility of transmission of the same deletions to their male offspring, who could also experience infertility. This fact is also significant because these men are at an increased risk for having recurrent miscarriages with their partner, and children with birth defects and learning disabilities.

### THE ROLE OF GENETIC COUNSELLING

Couples experiencing male factor infertility who are interested in pursuing assisted reproduction are encouraged to discuss genetic testing with their physicians or healthcare providers and to consider undergoing genetic counselling. Genetic counselling is a profession created to educate both couples and their care providers about genetic risks as they pursue assisted reproductive technologies, with attention

paid to the psychosocial impact of genetic information and infertility. Couples with infertility problems are part of a patient population with some of the highest identifiable genetic risks. They are in significant need of genetic counselling. There are three relatively common diagnoses that present clear genetic risks and warrant discussion and testing, viz. obstructive azoospermia, non-obstructive azoospermia and oligozoospermia.

To enable decision-making, patients need to be informed about the tests. If testing is performed and an abnormality is identified, professional genetic counselling should be offered, where patients are provided with an explanation of the cause of the genetic defect they have been identified with by a geneticist. More importantly, the consequences for the person tested, his future children and his family members are discussed and, if necessary, further counselling and testing for other family members is organised. The options for the treatment of infertility considering the genetic aspects, prenatal diagnosis or preimplantation genetic diagnosis (PGD) are also discussed.<sup>(48)</sup>

#### **Y CHROMOSOME MICRODELETIONS: IMPLICATIONS FOR ART**

Y chromosome microdeletion analysis should routinely be offered to all men with severe oligozoospermia or azoospermia. There are several considerations that support a routine assessment of Yq deletions. Firstly, a positive test would provide a firm diagnosis of the man's problem, which, for some couples with longstanding infertility, can help resolve stress, blame or feelings of guilt. Secondly, knowledge of the type of Yq deletion may assist the clinician in determining the best type of artificial reproductive technology (ART) treatment. Thirdly, couples should be offered this information, as they must understand that their male offspring will almost certainly be subfertile and require reproductive monitoring from the time of sexual maturation.<sup>(49)</sup>

Accordingly, men with Yq microdeletions are often (but not always) infertile, but many can still father children through ICSI using the few viable sperms present in semen or mature spermatids isolated directly from the testes. Modern sperm recovery techniques have made it possible to help men with both obstructive and non-obstructive azoospermia to achieve genetic fatherhood. In patients with obstructive azoospermia, viable spermatozoa from either the epididymis or the testes can be used for the ICSI procedure. Epididymal and testicular sperm recovery in combination with ICSI now offers azoospermic men the possibility of fathering their own genetic children.

#### **CLINICAL ANDROLOGY: STILL A MAJOR PROBLEM IN THE TREATMENT OF HUMAN MALE INFERTILITY**

With the introduction of ICSI, the treatment of male

infertility has been revolutionised. However, the transmission of microdeletions to sons via ICSI has recently been described. Thus, men with microdeletions will presumably transmit the deletion, as well as the related fertility problem, to their sons. Following genetic counselling about their Yq deletion, most couples still proceed with *in vitro* fertilisation using either the male partner's sperms or donor sperms.<sup>(50)</sup> In a small number of cases, couples have used PGD to select female embryos for transfer,<sup>(50)</sup> in an attempt to avoid passing on the genetic abnormality to their children, while other patients selected the option of adoption. Although genetic causes of infertility can now be identified in some patients, it is clear that in the future, genetic causes will be identified in a much larger number of patients than today and we may need to consider testing options other than those presently available. This implies that in the future, there will be a much greater overlap between reproductive medicine and genetics, and it is imperative that professionals working in these two fields collaborate closely to treat patients with infertility in the best possible way.

#### **CONCLUSION**

Male infertility is most likely the result of deletions and/or mutations of one or more of the myriad of genes required for spermatogenesis. Since the proposition some 24 years ago, that Yq harbours a possible AZF region, a multitude of studies have been conducted in search of the elusive factor. The introduction of molecular techniques has provided great insight into the genetics of infertility. Yet, despite recent advances, such as the isolation of several candidate genes, our understanding of the genetic regulation of spermatogenesis remains limited. In particular, we are still unable to establish the precise genotype-phenotype correlation between specific Y chromosome deletions and the various testicular histology patterns seen in infertile men.

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