GENETIC AND ENVIRONMENTAL FACTORS INVOLVED IN HUMAN MALE INFERTILITY: A REVIEW

SHALAKA S RAMGIR, VALSALA GOPALAKRISHNAN ABILASH*
Division of Biomolecules and Genetics, School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu, India.
Email: abilash.vg@vit.ac.in

ABSTRACT
As a World Health Organization (WHO) guidelines, infertility is the couple’s inability to conceive after 2 years of regular unprotected intercourse. The investigation in male infertility is assuming greater importance because approximately half of all infertility cases caused by male factors. Although previous studies suggest that many cases of male infertility have a genetic and environmental etiology to the condition, and the majority of cases are idiopathic. About 10-20% of azoospermic patients are showing the microdeletion in Y chromosome. In this deleted region, azoospermia factor (AZF) locus which is located in the Yq11 divided into the four regions as AZFa, AZFb, AZFc, and AZFd. In each of these regions a particular testicular histology and candidate genes have been found. The deleted in azoospermia gene family is also the most frequently deleted in AZFc region. Recently, not only Y chromosome, but X chromosome and some autosomal genes are also found in respect to male infertility. Frequent attacks on the naked mitochondrial DNA of sperm will responsible for oxidative damage or mutation to the mitochondrial genome and lead to male infertility. The introduction of molecular techniques, such as intracytoplasmic sperm injection, genomics, proteomics, metabolomics, has provided great perception into the genetics of infertility. Still, our understanding to find a correlation between genotype and phenotype in male infertility remains limited.

Keywords: Infertility, Azoospermia factor, Deleted in azoospermia, Mitochondrial DNA, Intracytoplasmic sperm injection, Genomics, Proteomics, Metabolomics.

INTRODUCTION
The WHO announced infertility as the third most serious disease in the 21st century; where 48.5 million couples worldwide suffers from this reproductive system disease. Infertility is defined as couples are unable to conceive after a period of 2-year, but many couples look around after a year for medical suggestions [1]. As a American Urological Association, approximately 15% of couples are unable to conceive after 1 year of unprotected intercourse. A male factor is solely responsible in about 20% of infertile couples and contributory in another 30-40%. Male infertility is a multifactorial disorder. The genetic etiology includes chromosomal abnormalities, mitochondrial DNA mutations, and endocrine disorders of genetic origin. The non-genetic causes include hypogonadism, testicular maldescent, and structural abnormalities in the male reproductive tract, infection, impotence, chronic illness, medication, and immunological conditions. Moreover also numerous environmental factors are correlated with male infertility as temperature [2], noise associated with manufacturing [3] radiation exposure [4], electromagnetic waves [5], and a variety of chemicals [6]. Until now, various risk factors like temperature [7], automobile driving time a day [8], air pollution [9], regional differences in residential population density [10], mumps [11], stress [12], and alcoholism [13] are responsible for male infertility. Much research remains to be performed on the topic of male infertility, as many cases still receive “unknown cause” diagnosis. This present review focused on the genetic factors such as microdeletions on Yq, X-linked genes and autosomal genes and environmental factors involved in the context of human male infertility and also, molecular techniques used in assisted reproductive technology.

EPIDEMIOLOGY AND PREVALENCE OF MALE INFERTILITY
The distribution of infertility due to male factor ranged from 20% to 70% and that the percentage of infertile men ranged from 25% to 12% [14]. The major difficulties, which exist in the diagnosis of male reproductive dysfunction, serve to complicate the understanding of the epidemiology and a prevalence of male infertility [15]. Prospective epidemiologic studies may help physicians to understand male infertility [16].

AETIOLOGY OF MALE INFERTILITY
Infertility is either primary, where couples not become pregnant after at least 1 year of unprotected sex, or secondary where couples are able to get pregnant only for one time, afterward they are unable. Primary and secondary infertility is found in 67-71% and 29-33% of patients, respectively. Male factor is responsible for approximately 50% of the cases; where 30% are responsible for male factors, and 20% are associated with male and female factors [1]. Male infertility has many causes from hormonal imbalances to physical problems, to psychological, and/or to behavioral problems. Moreover, fertility reflects a man’s overall health [17]. About two-third population of infertile men have a problem with making sperm in the testes, either low numbers of sperm are produced and/or the sperm that are produced do not work properly. And also sperm transport problems are found in about one in every five infertile males. Obstructions in the tubes leading sperm away from the testes to the penis can cause a complete lack of sperm in the ejaculated semen [18]. The percentage of various causes for male infertility presented in (Fig. 1).

Molecular causes of male infertility are depend on spermatogenesis, which is a complicated process, and it is influenced by 2000 genes, most of them present on autosomes and approximately 30 genes are located on the Y chromosome. While autosomal genes regulate spermatogenesis and genes located on Y chromosome are not taken part in general body function except the male reproductive processes. In one survey of infertile men where sample size is 9766, the incidence of chromosomal abnormalities was 5% × 6% and of cases, sex chromosomal abnormalities are found in 4% × 2% of cases, and autosomal abnormalities are accounted for 1% × 5% [20]. In previous studies, it is observed that causes for male infertility 1-2% are occurred by pre-testicular (hypothalamus/pituitary) factors, 30-40% by testicular factors, 10-20% by post-testicular (obstruction) and 40-50% by non-classifiable [21]. List of various causes of male infertility presented in Table 1.
Sperm defects
In many studies, it has been observed that male infertility is mostly due to sperm abnormalities. These abnormalities can be caused by congenital birth defects, disease, chemical exposure, lifestyle habits, and many other unknown factors. Classification of male infertility according to semen parameters and sperm abnormalities were presented in Table 2.

Azoospermia
It is present in 10-15% of infertile men. The main causes are a testicular failure and ductal obstruction [42]. Obstructive azoospermia: In this type of azoospermia; sperms are produced but not ejaculated due to a blockage in the sperm duct system. This condition observed in 7-51% of azoospermic men [43]. The most common reason is a vasectomy done to induce contraceptive sterility, [44] congenital (agenesis of the vas deferens as seen in cystic fibrosis), or acquired such as infection. Non-obstructive azoospermia - this type of azoospermia is due to failure of spermatogenesis within the testis. It is diagnosed in approximately 10% of infertile men [45]. It is caused by genetic factors, Y chromosome deletion (e.g. azoospermia factor [AZF]-c region), [46] karyotype abnormalities, radiation and toxins, medications, hormone imbalances, and varicocele. In one of the studies, it is observed that non-obstructive azoospermia is found in 10.7% of total population and obstructive azoospermia is found in 0.9% of cases [47]. Fig. 2 shows the percentage of various causes of infertility in couples and Fig. 3 represent the abnormal morphology of sperm.

Varicocele
Varicocele is defined as swelling of the veins that drain the testicle. It is the most common cause found in infertile males. This prevents the cooling of the testicle and leads to oligospermia and asthenozoospermia.
radiation, or chemotherapy for treatment of cancer can induce male fertility.

**Undescended testicles**
Some male patients, unable to descend their one or both testicles from the abdomen into the scrotum during fetal development and this cause decrease their fertility.

**Hormone imbalances**
Abnormalities in the endocrine system affect the male fertility. High levels of luteinizing hormone (LH) and follicle-stimulating hormone and low levels of gonadal steroids cause gonadal failure which is a cause of male infertility. High levels of LH cause hypergonadotropic hypogonadism (HH), which cause infertility.

Defects in vas deference: Illness or injury may damage the sperm ducts.

**Sexual intercourse problems**
Erectile dysfunction, premature ejaculation, and painful intercourse, abnormalities in structures of reproductive organs, such as hypospadias or psychological problems, may affect fertility.

**Celiac disease**
In this digestive disorder, patients are sensitive to gluten that is responsible for male infertility. After taking gluten-free diet, the male fertility may improved.

**Prior surgeries**
Vasectomy, inguinal hernia repairing, scrotal or testicular surgeries, prostate surgeries, large abdominal surgeries affect the sperm production.

**GENETIC CAUSES OF MALE INFERTILITY [49]**

**Chromosomal aneuploidy**
All studies investigating sperm aneuploidy levels in infertile men have demonstrated a three-fold increase in aneuploidy levels compared to their fertile males. Increases in sperm aneuploidy have been reported for all infertility phenotypes including oligozoospermia, asthenozoospermia, and teratozoospermia.

**Chromosomal translocations**
Nevertheless, carriers of balanced chromosomal translocations, while normal phenotypically, may experience reduced fertility, spontaneous abortions, or birth defects.

**Robertsonian translocations**
Robertsonian translocations involve chromosomes 13, 14, 15, 21, and 22. The results from various studies vary widely with reports of 3-36% unbalanced sperm.

**Reciprocal translocations**
Reciprocal translocations are seen in carriers, i.e. heterozygotes where studies have shown that 19-77% of spermatozoa are unbalanced.

**Chromosomal inversions**
As with chromosomal translocations, inversions can cause infertility, spontaneous abortions, and birth defects. In inversion carriers, it has been reported that unbalanced sperm ranges from 1% to 54%.

**Numerical sex chromosomal abnormalities**
Numerical sex chromosomal abnormalities in males are relatively common with Klinefelter syndrome (47, XXY) where the prevalence of 5% men with severe oligozoospermia and 10% with azoospermia. And in 47, XY syndrome also sperm defects has been observed with a prevalence of 1-2 in 1000 live births; However, they are fertile.

**GENETIC DISEASES (MONOGENIC AND MULTIFACTORIAL), WHICH ARE DIRECTLY OR INDIRECTLY ASSOCIATED WITH MALE INFERTILITY [17]**

**Cystic fibrosis**
It is an inherited condition which involves the lungs and pancreas, but it also cause for infertility with or without mild sinus problems. The most men with cystic fibrosis suffer from obstructive azoospermia because they born without a vas deferens which causes male infertility.

**Noonan syndrome**
It is an inherited condition where males suffer from an abnormal gonadal (testicular) function which leads to infertility.

**Myotonic dystrophy**
It is an inherited multisystem condition with underdeveloped testes and abnormal sperm production which leads to male infertility in some cases.

**Hemochromatosis**
In this inherited condition, iron storage problem occurred. Moreover, 80% of males with hemochromatosis have testicular dysfunction.

**Sickle cell disease**
It is an inherited condition which affects the production of hemoglobin. In these patients gonadal failure, erectile dysfunction, and menstrual issues were found which lead to infertility [50].

**Sex reversal syndrome**
A man carrying a genetic sex chromosome of female leads to azoospermia and other characteristics related to infertility.

**Androgen receptor (AR) gene mutations**
A man with a defect in receptors for testosterone but showing normal male karyotype leads to infertility.

**Immotile cilia syndrome**
In this condition, sperms are nonmotile with a normal sperm count.

**Other syndromes**
Some syndromic cases associated with male infertility are Kartagener syndrome - Sperm immotility, metabolic syndrome [51], Persistent Mullerian duct syndrome, Aarskog-Scott syndrome, Kears-Sayre syndrome, polyglanular failure syndrome (Types 1 and 2), Bardet-Biedl syndrome, Prader–Willi syndrome, deafness-infertility syndrome [53], non-insulin dependent diabetes mellitus (hypergonadism, increased scrotal temperatures, impaired spermatogenesis, decreased sperm concentration and motility, and increased sperm DNA damage), dyslipidemia - increased oxidative stress (OS) in the testicular microenvironment and/or excructant ductal system), Kears-Sayre syndrome - cryptoorchidism, pubertal delay, subnormal testicular volume, and low gonadotropin levels and others, which are yet to be investigated.

**MICRODELETION OF THE Y CHROMOSOME**
Microdeletions in the long arm of Y chromosome have been found at a much higher rate in infertile men than in fertile controls. Aberrations on the Y chromosome are currently found in approximately 15% of infertile males [53]. The high frequency of Y microdeletions suggests that the Y chromosome is susceptible to spontaneous loss of genetic material. 13% of azoospermic males, 1-7% of severely oligozoospermic males, 5% of males with severe primary testicular failure showed Y chromosome microdeletion [54]. Various studies focused on the “AZF locus” at Yq11 [1]. Gr/gr deletion in AZFc region is significantly associated with male infertility among Caucasians in Europe and the Western Pacific region [55]. Additional genes associated with spermatogenesis in men are RNA - binding motif protein (RBM), deleted in azoospermia (DMZ), SP0X, and testis specific protein; Y-linked (TSPY) which are

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located on Y chromosome, and if deleted they reduce the chances of fertility [56]. The instability of the Y chromosome may be related to a high frequency of repetitive elements clustered along the length of the chromosome [57]. De novo deletions of Yq are the most frequent event in chromosomal abnormalities of men which are arise from recombinations of long stretches of highly repetitive DNA sequences in the process of meiosis or early pre-implantation development [58]. The introduction of intracytoplasmic sperm injection (ICSI) as an artificial reproduction technique may allow the transmission of Y deletion to the next generation [59].

**MITOCHONDRIAL DNA MUTATIONS**

Mitochondrial genome is producing many essential compounds of the respiratory chain which have a great impact on sperm motility. Sperm needs high adenosine triphosphate (ATP) to travel through female reproductive tract which accomplish by sperm mitochondria [49]. Mitochondrial genes as COX II, ATPase 6 and 8 plays an important role but after mutation in these genes ATP production disturbed and spermatogenesis and sperm motility is affected. Therefore, the mutations in mitochondrial genome cause male infertility [60].

**ENVIRONMENTAL CAUSES OF MALE INFERTILITY**

Overexposure to certain environmental factors such as heat, chemicals, and toxins can reduce sperm production, and function. Specific factors include as following:

**Industrial chemicals**

High exposure to benzenes, toluene, xylene, pesticides, herbicides, organic solvents, painting materials, and lead may affect the sperm counts.

**Heavy metal exposure**

Lead or other heavy metals exhibition may cause male infertility.

**Radiation or X-rays**

Exposure to radiation may lower the sperm production, though it will often return to normal level, but if high doses of radiation given then sperm production may be permanently reduced.

**Exposure of high temperature to testicles**

Most commonly use of saunas or hot tubs can temporarily lower sperm count.

**OTHER CAUSES**

**Illegal drug**

To build up muscle strength and growth some anabolic steroids used which cause the testicles to shrink and to decrease sperm production. Cocaine or marijuana consumption also temporarily reduces the number and quality of sperms.

**Alcohol**

Drinking of alcohol may lower testosterone levels and also cause erectile dysfunction and lowers sperm production. Liver disease after taking of excessive alcohol may lead to fertility problems.

**Smoking**

Smokers may have a lower number of sperm count than non-smokers.

**Emotional stress**

Certain hormonal imbalances may occur by stress which is essential to sperm production. Severe emotional stress may lead to fertility problems such as, lower the sperm count.

**Weight**

Hormonal changes in obesity may reduce male fertility.

**Prolonged bicycling**

Prolonged cycling may reduce fertility due to overheating the testicles.

**MOLECULAR ASPECTS IN MALE INFERTILITY**

**Structure of Y chromosome**

The Y chromosome representing only 2-3% of the haploid genome, but it has around 107 genes and pseudogenes which has been mapped. Many of these are responsible for spermatogenesis and other male-related functions and deletion of any of these can result in infertility [61]. It has a high proportion of repetitive elements [62]. There is two pseudoautosomal regions, PAR1 on the short (Yp) and PAR2 on the long (Yq) arm of Y chromosome that recombine with their homologs on the X chromosome. The rest of the Y chromosome (~95%) is known as "non-recombining region" (NRY) or as "male-specific region." The NRY contains intrachromosomal repetitive elements that may be homologous to regions on X chromosome or Y chromosome [63]. The NRY region can be divided into euchromatic, centromeric and heterochromatic regions [64]. Y chromosome is divided into five broad sections - Pseudoautosomal boundary regions (PABY). Pericentric region on the short arm harboring the sex determining gene, euchromatic region (DYS1) cytogenetically known as Yq11 (subdivided into Yq11.21, Yq11.22, Yq11.23), is about 24 Mb on the proximal long arm, Heterochromatic Yq12 region (DYZ1, DY2Z) on the distal long arm contains 30 Mb and DYZ3 region that is critical for the survival and propagation of the Y chromosome [65]. Diagrammatic representation of genes and sequence tagged site (STS) markers present on Y chromosome is presented in Fig. 4.

**Microdeletion on Y chromosome and male infertility**

In humans, the Y chromosome is important for testicular differentiation and spermatogenesis. Recently, researchers found that observation of microdeletions on Yq11 in a high number of infertile men [67]. Previous studies found that interstitial microdeletions in Yq11 showed in 10-15% of idiopathic azoospermia and severe oligozoospermia, and this incidence is even higher in testicular diseases such as idiopathic Sertoli cell-only syndrome [68]. However in multiple non-overlapping spermatogenesis loci on Yq11 have been identified and that observed regions as AZFa, AZFb, and AZFc [69,70] including various genes essential for spermatogenesis. Later on, it is reported that the possibility of the presence of a fourth region, namely AZFd, between AZFb and AZFc. It has been reported that the deletion of a particular genes on AZF locus results in a characteristic phenotype because they are responsible for a particular stage in germ cell differentiation [71].

**Fig. 4: Gene location and STS markers on Y chromosome [66]**
CANDIDATE GENES IN AZFA REGION RESPONSIBLE FOR MALE INFERTILITY

DBY (DDX3Y)
DBY gene is located in the AZFa region, and it has important role in infertility because it is localized in the testis and is involved in the development of premeiotic germ cells [72]. In the study of the transcriptional activity of several AZF region genes found in men with Sertoli cell only syndrome (presence of sertoli cells in testes but a lack of sperms in the ejaculate [73] had reduced levels of DBY transcripts, but the other genes were transcribed normally. This finding suggests that DBY may play an important role in spermatogenesis, but further studies must be performed to replicate this result [74].

USP9Y
The USP9Y gene is also involved in spermatogenesis [75]. Shortening or deletion of USP9Y gene involved with azoosperma [76], oligozoosperma [77], or oligoasthenozoosperma [78]. However, it seems that this gene may involve in spermatogenesis process because it can be passed on to generations.

UTY
UTY is codes for a protein which is rich in tetratricopeptide repeats which might be include in the protein-protein interactions. The gene was mapped to the SC interval corresponds to AZFa region. Deletion analysis revealed that deletion of UTY is associated DBY [79].

CANDIDATE GENES IN AZFB RESPONSIBLE FOR MALE INFERTILITY

RBMY
RBMY gene is a major gene in the AZFb region where it has six copies located on the Y chromosome [80]. RBMY1 codes for RNA-binding protein [81], which is a testis-specific splicing factor expressed in the nuclei of premature forms of spermatozoa [72]. In a study, it was observed that RBMY1 expression was reduced in azoospermic men [82].

PRY
The PRY genes are participating in the regulation of apoptosis which is an essential process to remove abnormal sperm from the population of spermatozoa [72]. If both the RBMY and PRY genes are removed, spermatogenesis is arrested completely [83], this observation indicates that these genes are involved in fertility in the AZFb region.

CANDIDATE GENES IN AZFC RESPONSIBLE FOR MALE INFERTILITY

DAZ
DAZ is the prime candidate gene in the AZFc region. Several genes other than DAZ have been mapped to the AZFC region, including chromodomain Y 1 (CDY1). In men with azoospermia, oligosperma found with partial deletion in DAZ [72].

CDY1
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 [84].

BPY2, PRY, and TTY2
These genes are also located in AZFc region. The function of these genes is unknown, but they show similar characteristics like they have multiple copies on the Y chromosome, they expressed only in the testes, and they are Y specific. Deletions in these candidate genes are found, in 2-10% of azoospermic or severely oligozoospermic men [85].

Gr/gr deletions
The classical AZFc deletion, which removes 3.5 Mb between the b2/b4 amplicons, is the most common type of deletion. The frequency and pathological significance of these partial deletions are not yet clear, although recently, a novel Y chromosome 1.6-Mb deletion is derived as “gr/gr” deletion, and it specifically observed in infertile men with multiple degrees of spermatogenic failure [86]. The gr/gr deletion removes two copies of DAZ and one copy of CDY1 also several other transcription units in AZFc region. Since it is observed that passes from generation to generation, the gr/gr deletion results in subfertility rather than complete infertility. The deletion observed with numerous Y haplotypes, which suggests that it is 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.

CANDIDATE GENES IN AZFD RESPONSIBLE FOR MALE INFERTILITY

No candidate gene has been identified till now in AZFd region. However, recently it is observed that the deletion of DYS237 locus in AZFd region indicated that it has some important of genes which required for spermatogenesis [87]. Patients with microdeletions restricted to AZFd may present with mild oligozoosperma or even a normal sperm count associated with abnormal sperm morphology. STS markers like SY133, SY145, SY153, and SY152 are used for screening the AZFd region. Many variations in the junction of the euchromatic and heterochromatic regions have been deleted, which are reported from the STS marker (SY153) in AZFd.

OTHER GENES

TSPY
The TSPY gene is located on Yp, and it also has copies on Yq [74]. TSPY is expressed in the testis, and its protein has been detected in spermatogonia [88]. The TSPY gene can regulate the timing of spermatogenesis by signaling spermatogonia to enter meiosis [73]. Further investigation has to be done on TSPY to characterize its role in infertility because in a previous study it was seen that infertile men has variations in copy numbers of TSPY gene [89].

Autosomal DAZL gene
The DAZL gene has an autosomal homolog, i.e., DAZL which has location as 3p24. It is highly homologous to the DAZ gene about 83% of similarities in the coding region of the cDNA. Both DAZ and DAZL are the RNA-binding protein [90]. In one of the previous study, it is observed that 60% of idiopathic male infertility arises from autosomal recessive mutations [91]. A novel mutation, i.e., AG transition at nucleotide 386 in exon 3 of the DAZL gene, was identified in some infertile male patients. The mutation is located in the RNA-recognition motif (aa32-117) domain of the DAZL protein, and it will lead to change in Thr54 Ala (T54A) of DAZL protein [92]. There are, however, no reported instances of DAZL gene mutations among infertile men in India [93]. Whether DAZL plays a crucial role in spermatogenesis in humans also merits investigation.

X linked genes
Many X-linked genes are expressed in the testis [94] and are thought to be involved in gametogenesis. The AR gene is located on the Xq11.2-q12 and has four functional domains: the N-terminal transactivation domain, the DNA binding domain, the hinge region and the ligand binding domain [73]. It plays a role in meiosis and converts spermatocytes to spermatids [95]. In the recent study of infertile men, it is determined that approximately 2% of mutations are there in AR gene while the control one has none of them [96]. Mutations of the AR gene may also lead to androgen insensitivity syndrome [97].

TAF7L
The TAF7L gene has also been studied as a possible contributor to infertility in men. It is expressed in the testis and is related to the autosomal TAF7 gene, which is a transcription factor [88]. Transcription factor regulators show integral roles in spermatogenesis because they control the spatial and temporal factors which are important for accurate execution of the process [98-100].
In a recent study of hemizygous TEX11 mutations were a common cause of meiotic arrest; probably in the formation of crossovers during meiosis and azoospermia in infertile men. In a previous study, it was found that 99-kb hemizygous loss on chromosome Xq13.2 which involved three exons of TEX11. This mutation was identical in patients with azoospermia and shows a deletion of 79 amino acids within the meiosis-specific domain, SP022. Furthermore, subsequent mutation screening showed five novel TEX11 mutations as three splicing mutations, two missense mutations. These mutations are observed in 2.4% of azoospermia men [101].

**Kallmann Syndrome Interval Gene 1 (KAL1)**
KAL1 is located on the Xq arm which is responsible for migration of GnRH neurons, and it encodes anosmin-1 which is a cell adhesion molecule [102]. Defect in the migration of the GnRH neurons cause KS. It is an inherited disorder which can cause infertility in males, and it has both X-linked and autosomal genetic factors. KS is an idiopathic HH (HH) combined with anosmia or hyposmia. KAL1 gene deletions were found in KS patients [102,103].

**MITOCHONDRIAL DNA AND MALE INFERTILITY**

**DNA polymerase gamma (POLG)**
Sperm mitochondria play an essential role in functions of spermatozoa; therefore, genetic alterations to mitochondrial DNA may have correlation with normal fertilization. The important nuclear enzyme for mitochondrial polymerase activity is DNA POLG. The catalytic subunit of POLG is encoded by the POLG gene, which has been mapped to 16q15-q25 and it includes a CAG repeat region [104]. Mutations in the POLG gene affect the proofreading activity of the enzyme and results in the mutation of the mitochondrial genome and subsequently affect the ATP production. The association of a large stretch of CAG repeat is related with male infertility. Various studies have observed an association between different polymorphisms, mutations or deletions in the mitochondrial genome and sperm dysfunction [105]. One of the previous studies identified that specific mtDNA haplogroups are associated with asthenozoospermia.

**Mitochondrial DNA mutations in spermatozoa**
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 causes oxidative damage or mutation in the mitochondrial genome with pathological findings and results in male infertility [106]. The midpiece of the human sperm consists of 70-80 mitochondria in number, and there is one copy of mtDNA in each of them. About 85% of sperm samples contain large-scale mtDNA deletions of variable sizes, and most of them have 2-7 deletions of mtDNA in sperm. Among the mitochondrial deletions observed, the common deletion is in 4977 bp. Researchers have to study whether the mtDNA deletions are localized in the same mitochondria or in different organelles of spermatozoa which are harboring mutated mtDNA molecules. In one of the study, it was observed that a novel 2-bp deletion (8195 and 8196 nt) in the COII gene, which may result in a truncated protein [107]. In a previous study, it was found that two common substitutions at 9055 and 11719 in males responsible for reducing the sperm motility [108]. It has been found that high levels of A3243G mutant in mtDNA correlate with asthenozoospermia. The important role of genetic aberrations in etiology of human male infertility is increasingly recognized in research papers. While much remains to be find out in this fast-moving field. Much considerable progress has been made in the clinical delineation of genetic factors of male infertility. Diagrammatic representation of human mitochondrial DNA is presented in Fig. 5. Gene mutation and STS markers studied on male infertility in different population is presented in Table 3.

**NOVEL TECHNOLOGIES USED IN DIAGNOSIS OF MALE INFERTILITY**
Global approaches to the study of male infertility are currently being developed, which provides highly effective methods to diagnose and treat infertility. One of the main problems that geneticists face when they are relating a genotype to a specific infertility phenotype is that diversity in genetic backgrounds of different ethnic groups. Incorporating techniques as genomics, proteomics, and metabolomics into the field of ART develops a more approximate solutions in infertility.

**Microarray**
Microarrays are effective for identifying the gene expression profiles of infertile phenotypes [109]. Microarrays also examine the expression in spermatogenesis. Microarrays may be used to study the effect of hormones or growth factors on gene expression profiles. Microarray studies of gene expression produce various results to determine global gene expression patterns of RNA samples from the testis before it applied for clinical purpose.

**Genomic methods**
Genomic analysis is used to determine differentially transcribed genes [125]. Understanding of transcription regulation could help geneticists to discover the idea that how different expression patterns impact a patient’s fertility [109]. Characterization of a gene expression signature for human spermatogenesis may be used as a baseline marker [97]. These markers also correlate between infertile phenotypes and mRNA expression [109].

**Proteomics**
Proteomics determine the protein expression profiles of fertile and infertile men. Proteins are recognized by 2D electrophoresis and mass spectrometry techniques, and the results are utilized for creation of maps in the proteome [126]. Spermatozoa are novel for the study of protein expression because they do not have active transcription or translation. Further research in this field can continue after, the sperm proteome is fully defined and the factors of seminal plasma are identified. Recognition of protein biomarkers for male infertility will allow for unbiased comparison between fertile and infertile males, and it will also give us idea about pathophysiology of the diseases [127]. An advantageous characteristic of genomic and proteomic technology is that the results can be combined using other techniques such as Western blots, flow cytometry, and PCR.

**Metabolomics**
Metabolomics is used to measure the expression of metabolites, small biomarkers which indicate the functionality of a cell, and characterize them for certain diseases or physiological states [128,129]. Mass spectroscopy, nuclear magnetic resonance spectroscopy, and chromatography methods may be used to create profiles of metabolites. Pathway or cluster analysis is used to define subsets of metabolites that used to quantitatively characterize patients for diagnosis [130]. After identifying differences in the expression of metabolites in infertile
phenotypes, new methods can be developed which are inexpensive and noninvasive in diagnostic field. Furthermore, metabolomics used to identify biomarkers for OS, which are giving signals to examine the semen quality [128].

ICSI

Males with Yq microdeletions are mostly infertile, but many can still fertile through ICSI using the few viable sperms or mature spermatids isolated directly from the epididymis or testes. This sperm recovery technique helps the obstructive and non-obstructive azoospermic patients to achieve genetic fatherhood. However, by this technique transmission of microdeletions to sons has recently been described [131]. This observation implies that in the future, there will be a much greater overlap between reproductive medicine and genetics, and these two fields correlate very closely to treat patients with infertility in the best possible way.

CONCLUSION

Genes required for spermatogenesis has many mutations and deletions, which results in male infertility. Previous research findings suggest that genetic and some environmental factors are responsible for infertility of men. The molecular techniques used in ART have provided great insight into the genetics of male infertility. Through the efforts of researchers, it is possible to find relation between genetic mutations, environmental risk factors, and ethnic background to specific infertility phenotypes in men. Using this knowledge background, clinicians will be able to treat infertile males efficiently. Although much work still to be completed to fully determine the correlation between genotype and phenotype of male infertility.

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