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MEN'S HEALTH (A DABAJA, SECTION EDITOR)

Genetics of Male Infertility

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Abstract While 7 % of the men are infertile, currently, a genetic etiology is identified in less than 25 % of those men, and 30 % of the infertile men lack a definitive diagnosis, falling in the "idiopathic infertility" category. Advances in genetics and epigenetics have led to several proposed mechanisms for male infertility. These advances may result in new diagnostic tools, treatment approaches, and better counseling with regard to treatment options and prognosis. In this review, we focus on clinical aspects of male infertility and the role of genetics in elucidating etiologies and the potential of treatments.

Keywords Male infertility · Genetics · Epigenetics · Reproduction

Introduction

In the USA, about 15 % of the couples are infertile [1], and male factor is present in 50 % of the infertile couples [2]. Known genetic disorders are responsible for 15–30 % of male infertility cases, and genetic alterations yet to be discovered could well account for the majority of "idiopathic infertility" cases, which represent 30 % of all male infertility cases [3].

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Genetic male infertility disorders include chromosomal alterations, Y chromosome microdeletions (YCMD), gene mutations, and epigenetic disorders. The use of advanced reproductive techniques, such as microdissection testicular sperm extraction (microTESE) combined with in vitro fertilization techniques, preimplantation genetic diagnosis (PGD), and screening (PGS), may overcome some of these problems, but there is always a risk of transmission of the parental diseases to the offspring. Interest in this field has significantly increased as specialists try to gather data that could be useful in the management and counseling of these couples. In this article, we review the current literature regarding the genetic alterations linked to male infertility.

Chromosomal Alterations

Infertile men have higher incidence of chromosomal alterations, especially aneuploidy, with incidence ranging from 2 to 15 %, depending on the severity of spermatogenic impairment [4]. Chromosomal recombination during meiosis is crucial not only for evolution, but also for correct segregation of chromosomes during spermatogenesis. Thus, recombination failure is linked to sperm aneuploidies. Not surprisingly, infertile men have a higher rate of recombination failure [5], and sperm aneuploidy, even when classical semen analysis parameters, is normal [6••]. While autosomal chromosomes can undergo recombination along their entire length, X-Y chromosomal pairing and recombination is restricted to the small homologous pseudoautosomal regions [7]. This explains why sex chromosomes aneuploidies are the most frequently found genetic abnormalities in men. Other chromosomal alterations that can cause infertility include translocations and inversions.

Klinefelter Syndrome

Klinefelter syndrome (KS) is the most common genetic cause of male infertility, found in 1/600 of all men, 0.6 % of severely oligospermic men, and in 11 % of men with non-obstructive azoospermia (NOA) [8•, 9]. Nondisjunction during meiosis I is the origin of the extra X chromosome, which has been shown to be paternal-derived in 60 % of the cases. Association with increased paternal age is controversial, while maternal age is considered a risk factor [10]. In mammals, a major part of the extra X chromosome is inactivated, and only 15 % of the genetic content escapes silencing. However, in patients with KS, this process is disturbed, resulting in an excessive genetic output that impairs androgen production and spermatogenesis [8•, 10].

Clinical features of KS include infertility, hypergonadotrophic hypogonadism, and cognitive disorders. The phenotype spectrum is ample, varying with the extent of genetic inactivation and the presence of mosaicism [8•, 11]. A consistent finding in men with KS is progressive degeneration of germ cells (GCs) and Sertoli cells (SCs), mainly after puberty [12]. The mechanisms leading to testicular degeneration are still unknown, but overexpression of X chromosome genes, some of them related to inflammatory pathways and blood testicular barrier (BTB) structure, malfunction of follicle stimulating hormone (FSH) receptor, and increased aromatase activity may have a role [13–16].

Androgen receptor (AR) gene inactivation also contributes to the pathophysiology of KS. Located in the Xq chromosome, the AR gene contains a critical region of CAG-nucleotide repeats located in the exon 1, and the length of this region is inversely related to the receptor activity. Consequently, inactivation of the AR gene with a shorter or longer stretch of CAG repeats may be related to the severity of the syndrome [17]. Furthermore, a recent paper reported that testosterone (T) production by Leydig cells (LCs) is normal or even increased in men with KS [18]. This evidence suggests decreased release of T into the bloodstream associated to the lack of responsiveness of the AR.

Even with progressive testicular degeneration, sperm retrieval rates with microsurgical techniques (microTESE) are high (70 %) in men with KS [19], and the use of the retrieved sperm with in vitro fertilization techniques seems to be safe [20]. The production of normal sperm by these men is probably due to niches of undisturbed spermatogenesis composed by either a few GCs with normal karyotype, or by some 47,XXY GCs that are able to go through meiosis and produce sperm with normal karyotype (23,XY or 23,XX) [21, 22].

Other Sex Chromosomal Aneuploidies

47 XYY karyotype is found in 1/1000 of live births and is the second most frequent aneuploidy of sex chromosomes [23]. The extra Y chromosome originates from nondisjunction during meiosis II. These men may be phenotypically normal, but high stature, clinodactyly, hypertelorism, cognitive impairment, aggressive behavior, and infertility are some of the characteristics that can be found. When compared with the general population, XYY men have higher incidence of asthma, seizures, tremor, and autistic spectrum disorder [23]. Testosterone levels are normal or elevated [24]. XYY men with impaired spermatogenesis present with elevated FSH, and sperm analyses shows azoospermia or severe oligospermia [25]. Sertoli cell-only syndrome (SCO) and maturation arrest (MA) are common testicular biopsy findings [26]. There is an increased incidence of chromosomally abnormal spermatozoa in the semen of men with 47,XYY syndrome, especially sex chromosome disomies [27, 28].

46 XX male is a rare chromosomal abnormality, found in 0.9 % of the azoospermic male [29]. Delayed puberty, gynecomastia, and infertility are the most common clinical findings, whereas hypospadias, cryptorchidism, and genital ambiguity are rarely reported. There are two variants of this condition. The first one, responsible for 80–90 % of the cases, is caused by the translocation of the sex-determining region Y (SRY) gene ($SRY^{+}XX$ males). All the men with this variant are azoospermic, but usually with normal male phenotype [30]. The second variant is the $SRY^{-}XX$ males, in which no copy of SRY is found. In these cases, the male phenotype is due either to mutations in autosomal or X-linked genes involved in the sex-determining cascade, such as SOX9 and DAX1 genes, which substitutes the SRY. These patients are more likely to have incomplete masculinization [31, 32].

45X/46XY mosaic is another rare condition with a broad spectrum of phenotypes. 45X/46XY men may present with impaired gonadal development, intra-abdominal testes, infertility, and hypospadias. Another characteristic is the higher predisposition to gonadoblastomas and dysgerminomas. Sperm analysis of 45X/46XY oligospermic men showed higher frequency of aneuploid sperm, which suggests an increased risk of producing offspring with chromosome abnormalities [33, 34].

Robertsonian Translocations

Robertsonian translocations (ROB) occur when two acrocentric chromosomes (i.e., chromosomes 13, 14, 15, 21, and 22) fuse their long arms, leading to the loss of the genetic material on the short arms. ROB are the most common structural abnormalities, found in 1/1000 newborns and in 0.9 % of the infertile men [29]. Despite normal phenotype, men with ROB may have impaired spermatogenesis because of faulty segregation of the fused chromosomes as well as interferences on the pairing and segregation of other chromosomes. The affected men show higher incidence of sperm aneuploidy [35], and there is a risk of passing on the translocation to offspring. Therefore, their sperm chromosomal composition should be

analyzed; the couples should have proper genetic counseling, and preimplantation genetic diagnosis must be offered [36].

Autosomal Inversions

Autosomal inversions are structural chromosomal derangements that do not lead to genetic material loss. Chromosome 9 inversions are the most relevant for male infertility, being found in 3–5 % of the infertile men [37, 38]. Male carriers of chromosome 9 inversions may show azoospermia, oligospermia, asthenozoospermia, or normozoospermia. They also have a higher incidence of sperm aneuploidy [39].

Y Chromosome Microdeletions

The euchromatin zone of the long arm of the Y chromosome (Yq11) houses the AZF (azoospermia factor) region, which contains genes critical for spermatogenesis. These genes are divided in three groups based on their location: AZFa, AZFb, and AZFc (Fig. 1) [40]. Microdeletions located in these zones may impair fertility and are present in 10 % of men with NOA and in 5 % of those with severe oligospermia, but the incidences and phenotypes vary geographically and ethnically [41–43].

Accounting for 60 % of all YCMD, the AZFc group is located in the distal aspect of Yq11 [44, 45]. Several genes are located in the AZFc group, and the DAZ (deleted in azoospermia), a family of four genes implicated in spermatogenesis, has been the most studied [46]. The arrangement, similarity, and the huge size of its amplicons, repetitive copies of nucleic acid sequences, are responsible for the relative high incidence of de novo deletions via homologous recombination (HR) in this group [47]. The most frequent deletion affecting the AZFc group involves the amplicons b2 and b4 (b2/b4), and removes eight genic families, including the DAZ family. Smaller partial deletions also exist and may happen either via HR, such as "b1/b3," "b2/b3," and "gr/gr", or via non-homologous recombination, such as P3a, P3b, P3c, and P3b [45, 48].

The clinical and histological presentation spectrum is wide, but, in general, AZFc deletions are compatible with pockets of spermatogenesis. Men with AZFc deletions usually present with azoospermia or, more often, severe oligozoospermia. Histological patterns vary from SCO, to (MA) and hypospermatogenesis (HS). When microTESE is used, viable sperm can be harvested in approximately 50–60 % of the azoospermic men [41]. Complete AZFc deletions could cause Y-chromosome loss and lead to 45X/46XY karyotype with Turner stigmata or sexual ambiguities. To avoid the transfer of 45X0 embryos, PGD should be offered to these couples [49]. In addition, since YCMD will be transmitted, and might also increase in extent, male offspring is expected to show the same or worse degree of spermatogenesis impairment as their fathers. This fact should be taken into consideration during genetic counseling.

The AZFb group is affected in 15 % of YCMD cases. It contains two genes important to spermatogenesis, the RBMY1 and PRY genes. The first is a testis-specific splicing factor expressed in the nucleus of spermatogonia, spermatocytes, and round spermatids, and the second is involved in the regulation of GCs apoptosis [45]. Complete deletions of this zone (proximalP5/P1) are massive, perhaps the largest in human genome. Homologous and non-homologous recombinations, as well as other unknown factors, are involved in these events [50]. Combined AZFb + AZFc deletions may occur, since AZFb overlaps AZFc by 1.5 Mb, and are, indeed, more frequent than isolated AZFb deletion [41, 51].

Men with complete AZFb or AZFb + AZFc deletions present with azoospermia, and testicular biopsy usually shows

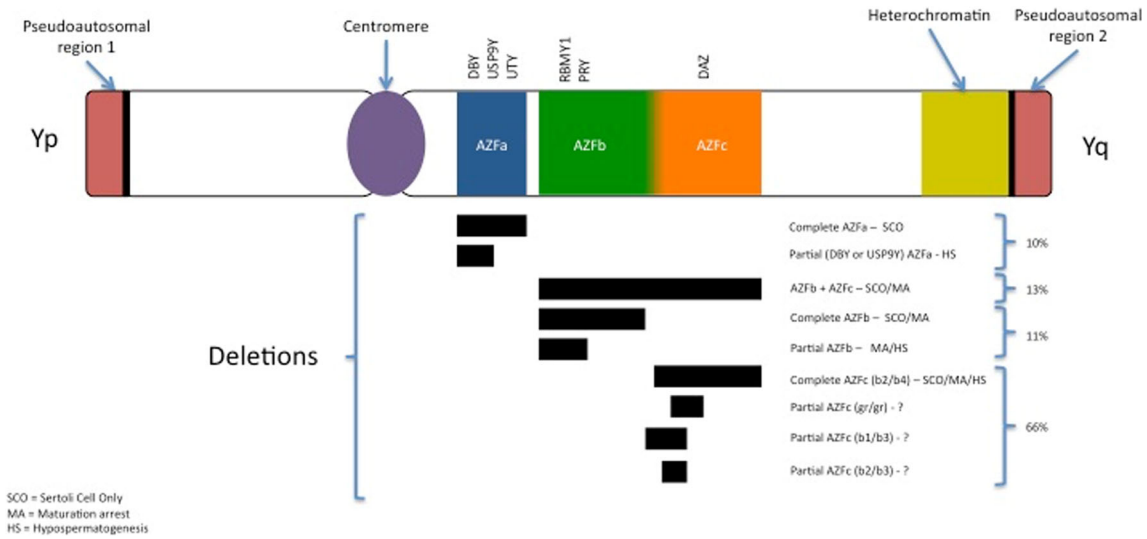

Fig. 1 Y-chromosome microdeletions [41, 43, 51, 148]. Reprinted from Seminars in Cell and Developmental Biology 2016. Neto FTL, Bach PV, Najari BB, Li PS, Goldstein. Spermatogenesis in Humans and Its affecting factors. Article in press .with permission from Elsevier

SCO or diffuse early MA. Currently, these patients must use donor sperm or adoption [41].

Situated near the centromere, the AZFa group has about 1100 kb and contains three main genes: DBY, USP9Y, and UTY. The DBY gene acts as a spermatogenic regulator during the earliest stages (i.e., spermatogonia), while the other two are, apparently, not crucial for male fertility [52, 53]. AZFa deletions have been shown to occur via intrachromosomal recombination between flanking repeats [54].

Complete deletion of AZFa is rare (3 %) and carries the poorest prognosis of all YCMD. Invariably, these men have azoospermia and SCO, and no sperm is found using microTESE. Therefore, these patients should not be submitted to invasive sperm retrieval procedures, but referred for donor sperm or adoption. In contrast, men with partial AZFa deletions often have HS and present with severe oligospermia or cryptozoospermia [41, 51]. We expect that advances in genetic engineering will allow us to induce progression through meiosis in stem cells, producing spermatozoa, and helping men with complete AZFa, AZFb, and AZFb + c deletions to father offspring in the future.

Single Gene Mutations

More than 3000 genes have been associated with spermatogenesis [55], but despite increased efforts by genetic labs worldwide, less than 0.01 % of these genes have been evaluated in infertile men [56]. However, with the decreasing costs of next-generation sequencing, we expect that these genetics tools will achieve widespread use for both, research and clinical purposes, and accelerate the knowledge gathering process in this area. This section will focus on single gene mutations that have clinical importance for human male infertility.

Cystic Fibrosis Transmembrane Conductance Regulator Gene Mutation

The cystic fibrosis transmembrane conductance regulator (CFTR) gene is located on chromosome 7 and encodes an anion channel critical for salt homeostasis of several epithelial tissues such as the lung and pancreas [57]. More than 1950 CFTR mutations have been identified so far [58]. These mutations differ regarding their molecular mechanisms and their impact in the channel function [45]; therefore, the phenotype spectrum of carriers is wide. Patients with severe mutations develop cystic fibrosis (CF), the most common lethal genetic disease in Caucasians, with an approximate incidence of 1 in every 3500 newborns [59], while men carrying mild mutations may present only with congenital bilateral absence of the vas deferens (CBAVD).

CBAVD is found in about 1 % of the infertile men, and in up to 25 % of men with obstructive azoospermia [60]. Most patients with CBAVD have only the caput and corpus of the epididymis present, with non-palpable vasa and epididymal cauda. A few patients may have palpable scrotal vasa, that, in most of the cases, end blindingly in the retroperitoneum, without communication with the ejaculatory ducts [61]. The seminal vesicle and ejaculatory ducts may also be absent in these men [62]. A recent meta-analysis [60] found that 78 % of men with CBAVD have at least one CFTR mutation, and 46 % have two mutations. The most common mutant alleles are the F508del, which is also the most common allele in patients with CF, the 5 T, and the R117H. The most frequent heterozygous genotypes are the F508del/5 T and F508del/R117H. F508del/F508del genotype is not frequently found in men with isolated CBAVD, since it implicates in a severe impairment of CFTR protein function, leading usually to CF. Ethnicity influences the frequency of mutations, with non-Caucasian showing lower incidence of two mutations, as well as lower incidence of F508del allele. Other authors reported that in men with CBAVD that were diagnosed with only one CFTR mutation, a search for rare mutations with "regional impact," large genomic rearrangements, and point mutations within the entire CFTR coding region revealed a second mutation in 40 % of them [63].

Even though the genetic link between CFTR mutations and CBAVD is irrefutable [60], the molecular mechanisms behind it are still unclear. Theories about the cause-effect relationship include obstruction of the genital ducts by mucus and secretory protein accumulation during early development and subsequent ductal atrophy [64], direct impact in the development of the Wolffian duct [65], and decreased activity of the Wnt/β-catenin signaling system, which is critical for Wolffian duct differentiation [66], but none of these theories have been tested yet.

In addition, there is a debatable association between CFTR mutations and poor sperm function [58, 67–70]. Several theories of how these mutations could affect spermatogenesis and sperm maturation have been proposed [66], but more studies are needed first to better clarify this association, and them to explain its mechanisms.

Men with CBAVD are good candidates for sperm retrieval from the epididymis or testis coupled with intracytoplasmic sperm injection (ICSI) [71, 72]. Genetic testing for CTFR mutation is mandatory, not only for patients with CABVD, but also for their female partners, since up to 1 out of 25 individuals are asymptomatic carriers of a mutation [59]. Moreover, the mutation panel of the test should take in consideration the couple's ethnic background. The offspring of a carrier couple has a high risk of develop CF; thus, genetic counseling and preimplantation genetic diagnosis are indicated based on the mutations found. Furthermore, a

search for rare mutations should also be considered if the female partner is found to be a carrier [63].

Kallmann Syndrome

Kallmann syndrome (KLS) has an incidence of 0.2 % [73] and an estimated 5 M:1 F sex ratio [74]. KLS is mainly characterized by hypogonadotropic hypogonadism, delayed puberty, infertility, and defective sense of smell (anosmia or hyposmia) [75]. The syndrome has genetic and phenotypic heterogeneity, and several genes have been associated with this condition [76]. Kallmann syndrome 1 (KAL1) and the fibroblast growth factor receptor 1 (FGFR1) are the two most studied KLS genes.

KAL1 (now denoted ANOS1) was the first gene associated with KLS. KAL1 is an X-linked gene that encodes a cell adhesion protein of the extra cellular matrix (anosmin-1). Anosmin-1 acts as a chemoattractant and plays an important role in the migration of gonadotropin-releasing hormone (GnRH) neurons from the olfactory placode to the preoptic area of the hypothalamus during embryologic development; thus, mutations affecting the KAL1 gene cause migration arrest of GnRH-1 neurons [77]. In this variant, female are usually carriers, and other phenotypic features include renal anomalies, mirror movements, and neurogenic deafness [78, 79]. The prevalence of KAL1 mutations varies from 14–100 % in the familial cases, and from 11–33 % of the sporadic cases [80, 81].

FGFR1 gene is located on the chromosome 8, and its encoded receptor is part of a signaling pathway implicated in the olfactory system and GnRH neuron ontogeny [82]. Loss-of-function mutations of this gene are responsible for the autosomal dominant variant, which is found in 10 % of KLS cases [74], and linked to extremities malformations [83] and midline defects, such as cleft palate and dental agenesis [78].

Since men with KLS lack GnRH releasing neurons, endocrine evaluation typically reveals undetectable levels of luteotropic hormone (LH) and FSH, and very low T. These men with failure to complete puberty, except in rare cases of spontaneously reversible KLS [84], have incomplete spermatogenesis due to the low levels of intratesticular testosterone and absent FSH.

Genetic testing can be used for diagnosis and should be guided by the inheritance pattern and by the presence of additional phenotypic features [85, 86]. Genetic diagnosis is also useful for prognosis and for couple counseling regarding the risks for their offspring and the use of PGD.

After the diagnosis is made, hormonal treatment should be planned to achieve two main objectives: inducing virilization and normal development and improving the fertility status. There are several gonodotropin-based regimens that can be used in patients willing to maintaining fertility, and success to promote normal puberty and full spermatogenesis with fertility is very high [85].

Testis-Expressed 11 Gene Mutations

The testis-expressed 11 (TEX11) is a newly identified germ cell-specific gene located in the X chromosome [87]. TEX11 encodes a protein that regulates homologous chromosome synapses, recombination, and double-strand DNA break repair [88, 89]. In animal models, TEX11 mutations have been associated to MA at primary spermatocyte stage and azoospermia [88, 89]. These mutations were also found in 2–7 % of the azoospermic men and were associated with spermatocyte apoptosis, MA, and azoospermia [90, 91]. However, it is still unclear whether men with these mutations may have niches of undisturbed spermatogenesis in their testes, and thus be amenable to microsurgical sperm retrieval (microTESE) [90].

Dpy19l2 Gene Mutations

Acrosome development is a key stage during spermatogenesis, and disturbances during this process could lead to globozoospermia, a rare infertility condition characterized by round-headed spermatozoa that are unable to penetrate and activate oocytes [92]. DPY19L2 gene is located in chromosome 12 and encodes a protein of the inner nuclear membrane that participates in the acrosome development [93]. DPY19L2 gene mutations are the most common cause of globozoospermia, while SPATA16 and PICK1 mutations are also associated with this phenotype [94]. The outcomes of conventional intracytoplasmic sperm injection (ICSI) are poor in these men, since their sperm cannot active the oocyte. Fortunately, the use of ICSI coupled with assisted oocyte activation has resulted in live births [95]. Men with complete or almost complete globozoospermia should be tested for DPY19L2 mutations, and if mutation is found and the parents are related, the woman should be tested, and the couple should have genetic counseling [96].

Epigenetic Factors

Epigenetics is the study of several processes that alter gene expression without changing the DNA sequence. These processes include DNA methylation, post-translational histone modifications, chromatin remodeling, and microRNAs regulation. They can vary among different types of cells, tissues, organs, sex, species, and developmental stages. Epigenetic factors may be carried through generations or may be reversible [97]. Alterations of epigenetic factors are implicated in the pathophysiology of several male infertility conditions and are probably responsible for some cases of "idiopathic" male infertility. New diagnostic and therapeutic tools will likely accrue from research in this field.

Another key aspect of epigenetics regarding reproductive medicine is the association between the use of assisted

reproductive technologies (ART) and pathologies related to genomic imprinting, such as Prader-Willi, Beckwith-Wiedemann, and Angelman syndromes. This association may be explained by the use of defective sperm with incomplete reprogramming, or epigenetically imperfect oocytes arising from superovulation. Other cause may be ART procedures performed at the time of epigenetic reprogramming [97–100].

In addition, epigenetic process may be the mechanism by which several diseases and conditions, such as obesity and environmental exposure, affect spermatogenesis and influence the offspring [101–103]. However, further studies are needed to clarify these associations.

DNA Methylation

DNA methylation is the addition of a methyl radical to cytosine-guanine dinucleotides (CpG) by DNA methyltransferases. CpG islands, areas of DNA with high content of CpG, have been found near promoters, and hypermethylation of CpG islands is linked to gene suppression, while hypomethylation is associated with gene expression [99, 104]. Epigenetic reprogramming of GCs by widespread erasure of DNA methylation followed by de novo methylation is a relevant event for spermatogenesis. There are two stages of epigenetic GCs reprogramming, one during the gonadal development, and another one during adult life, establishing a male germ line pattern of DNA hypomethylation. Not surprisingly, aberrant global DNA methylation, as well as aberrant DNA methylation at specific sites, has been associated with poor quality human sperm and decreased fecundity. However, the cause-effect relationship between DNA methylation and fertility remains unclear [105–111].

Post-Translational Histone Modifications

Histone methylation, acetylation, phosphorylation, ubiquitylation, and sumotylation also modulate gene expression. Post-translational modifications take place in amino acid residues in the N-termini of histone tails, and several enzymes are involved in the process. The combination among different sites and radicals will determine the final effect, gene activation, or suppression [97, 112–114]. Animal studies have shown that several subtypes of histones, such as H1, H2, H3, and H4, undergo post-translational modifications [115], and the few human studies about this topic have described an association between altered H3 methylation and poor sperm quality [116, 117].

Chromatin Remodeling

The manner by which DNA segments are packed around histones determines whether or not genes are available for transcription. DNA segments found in heterochromatin are tightly packed, and, thus, silent, while the loose DNA segments of euchromatin are usually transcriptable. Therefore, chromatin remodeling may activate or inhibit gene expression. The exact remodeling mechanisms are still unknown, but ATP-dependent chromatin remodeling complexes appear to participate in the process [118, 119].

In order to fit into the sperm head, the DNA content of the male GCs should be neatly packed in a very small volume. During spermatid stages, the replacement of 80 % of the histone content by protamines type 1 (P1) and type 2 (P2) via hyperacetylation of histone H4 is necessary to form supercoiled structures named toroids, increasing DNA packing and protection [120]. The degree of histone-protamine replacement has been correlated with the fertilizing capacity of the sperm, and decreased levels of H4 hyperacetylation were demonstrated in men with MA [121–123]. In addition, residual histone-bound DNA content is crucial for sperm function and early embryo development. The P1/P2 ratio also affects fertility. Fertile men usually have an equal proportion of P1 and P2 [124], and altered P1/P2 ratio has been linked to increased DNA fragmentation, decreased sperm function, reduced pregnancy rates, and found in some male infertility conditions [125–128].

MicroRNAs

MicroRNAs (miRNAs) are a class of short (20–23 nucleotides) single-stranded non-coding nucleotides, and constitute one of the most abundant ribonucleoprotein complexes in the cell. miRNAs modulate the expression of several protein-coding genes. Their mechanisms of action are still under debate, but may include direct destruction of targets mRNAs, translation repression, and other indirect pathways to inhibit protein synthesis [129–131]. Due to the fact that the expression of miRNAs varies among different developmental stages, tissues, and diseases, specific expression patterns could be linked to specific pathologies and, therefore, be used as diagnostic and therapeutic tools [132].

Regarding spermatogenesis, several animal studies showed that GCs miRNAs, many of them stored in the cromatoid body, are implicated in the regulation of apoptosis, proliferation, and differentiation. This post-transcriptional regulation is essential because GCs are transcriptionally silent during certain stages of spermatogenesis. Alterations of miRNAs expression patterns could impair spermatogenesis and might explain a number of “idiopathic” male infertility cases [133–135].

Recently, efforts have been directed to associate specific miRNAs expression patterns in the seminal plasma with human testicular histopathologic patterns and clinical findings, with the idea of creating new diagnostic tools to assess human male fertility [136]. So far, 1881 human miRNAs have been described (www.mirbase.org)

Table 1 Lists of the most studied miRNAs in infertile men [132, 149–152]

Name	Function	Expression	Findings associated
miR-34 family	p53 tumor suppressor network	Down	NOA and oligospermia
miR-122	Suppresses the transcription of transition protein 2	Down	NOA
miR-19b	Inhibition of apoptosis	Up	NOA
Let-7a	Cell proliferation	Up	NOA
miR-181a	Regulation of T cell sensitivity	Down	NOA
		Up	Oligospemia
miR-146b	Regulation of apoptosis	Down	NOA
		Up	Oligospermia
miR-513a-5p	Regulation of apoptosis	Down	NOA
		Up	Oligospermia
miR-509–5p	Regulation of apoptosis	Down	NOA
		Up	Oligospermia
miR-374b	Oncogene	Down	NOA
		Up	Oligospermia
miR-141	Regulation of cell cycle	Up	NOA and Oligospermia
miR-429	Unclear	Up	NOA and Oligospermia
miR-202-5p	Unclear	Down	NOA
miR-7-1-3p	Regulation of cell cycle	Up	NOA

[137], and several have recognized impact on male fertility. Table 1 lists some miRNAs that are increased or decreased in infertile men. For a good review of this topic refer to [110••].

DNA Damage

DNA damage is caused by single-strand and double-strand breaks that are left unrepaired. Strand breaks are naturally formed during meiosis 1, to allow recombination, and during spermiogenesis, to allow unwinding of the nucleosomal structure and again to avoid supercoiling [138]. They might also be formed by the release of reactive oxygen species (ROS) due to a variety of causes, such as incomplete apoptosis [139], prolonged epididymal transit [140], environmental chemical exposures, varicocele, diabetes mellitus, and others [141]. Even though DNA strand breaks may be repaired during spermatogenesis and early embryogenesis [138], the repair mechanisms may be insufficient in the setting of massive DNA damage.

During spermatogenesis, GCs with high content of irreparable DNA fragmentation may be directed to apoptosis [142], but some may escape it and become defective sperm [138]. Both mechanisms lead to male infertility, and increased DNA fragmentation has been associated with decreased pregnancy rates, both natural and with ART [143, 144, 145•, 146]. Since conventional semen parameters do not correlate well with the DNA fragmentation status of the sperm [147], specific methods to assess sperm DNA damage should be used to correctly diagnose these men, and help them to choose the best treatment option.

Conclusion

Advances in genetics and epigenetics are steadily finding etiologies in "idiopathic male infertility". Genes located on the X chromosome and autosomal chromosomes are increasingly implicated as causes of male infertility conditions. In the same way, newly discovered epigenetic factors have been shown to influence male gamete differentiation and function. Despite all the new knowledge, the exact molecular mechanisms of how genetic and epigenetic alterations affect human spermatogenesis are still unclear. Future research in this area will continue to provide clinically useful tools for diagnosis, treatment, and counseling of affected couples.

Compliance with Ethical Standards

Conflict of Interest Filipe Tenorio Lira Neto, Bobby Baback Najari, Philip Shihua Li, and Marc Goldstein declare no potential conflicts of interest.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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