Towards Post-Meiotic Sperm Production: Genetic Insight into Human Infertility from Mouse Models

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Abstract

Declined quality and quantity of sperm is currently the major cause of patients suffering from infertility. Male germ cell development is spatiotemporally regulated throughout the whole developmental process. While it has been known that exogenous factors, such as environmental exposure, diet and lifestyle, et al, play causative roles in male infertility, recent progress has revealed abundant genetic mutations tightly associated with defective male germline development. In mammals, male germ cells undergo dramatic morphological change (i.e., nuclear condensation) and chromatin remodeling during post-meiotic haploid germline development, a process termed spermiogenesis; However, the molecular machinery players and functional mechanisms have yet to be identified. To date, accumulated evidence suggests that disruption in any step of haploid germline development is likely manifested as fertility issues with low sperm count, poor sperm motility, aberrant sperm morphology or combined. With the continually declined cost of next-generation sequencing and recent progress of CRISPR/Cas9 technology, growing studies have revealed a vast number of disease-causing genetic variants associated with spermiogenic defects in both mice and humans, along with mechanistic insights partially attained and validated through genetically engineered mouse models (GEMMs). In this review, we mainly summarize genes that are functional at post-meiotic stage. Identification and characterization of deleterious genetic variants should aid in our understanding of germline development, and thereby further improve the diagnosis and treatment of male infertility.

Key words: Spermiogenesis, spermatogenesis, infertility, genetically engineered mouse model (GEMM), oligoasthenoteratozoospermia (OAT)

1. Introduction

Infertility is a rapidly rising concern among couples at reproductive age worldwide. Currently, around 6% of the married population among 15-44 years of age suffer from infertility [1]. It has thus become a severe health problem that elicits many social consequences. Almost 50% of fertility-related problems are caused by male factors [2], such as Y-chromosome microdeletions, Klinefelter syndrome, obstruction and genetic abnormalities [3]. In the clinics, some abnormalities can easily be screened by common cytogenetic and biochemical techniques available in most reproductive centers. However, nearly half of cases of male infertility are idiopathic and the genetic variants responsible for the compromised male fertility remain elusive.

In mammals, testis is mainly composed of tortuous, elongated seminiferous tubules in which spermatogenesis is taking place along the well-organized epithelia [4]. In general, the production of competent sperm after birth in mammals necessitates three successive processes: (i) mitotic proliferation of spermatogonial stem cells
(SCCs) and subsequent differentiation into advanced spermatogonia (differentiated spermatogonia); (ii) meiotic division: one-time duplication of chromosomes followed by two times of cell division; (iii) spermiogenesis, known as haploid spermatid development, is a process whereby the round spermatids morphologically transform into nuclei-condensed spermatozoa. These normal-looking sperm must be released from seminiferous tubules and transit through further convoluted epididymis to undergo functional maturation in order to be fertilization-competent [5].

At haploid stage, probably the most characteristic feature that distinguishes spermatid development from other somatic cell differentiation is the unique cellular structures formed, such as the acrosome and sperm tail [6]. At the molecular level, spermatid chromatin undergoes drastic chromatin remodeling and genome-wide histone removal followed by protamine deposition, a process termed as histone-to-protamine transition, leading to the production of highly condensed sperm heads with much less cytosolic contents. It has thus been conventionally thought that sperm function as a “carrier” that solely delivers the paternal genetic DNA into oocyte which initiates fertilization [7]. Recent progress in the field argued that the rich non-coding RNA molecules buried inside the sperm nucleus execute a significant impact on the subsequent development in the offspring epigenetically (i.e., independent of the DNA sequence) [8].

Therefore, understanding the mechanisms by which gene expression is specifically regulated to facilitate the spermatid elongation and genome-wide re-organization prepared for the sperm-egg fertilization, has significant implications for reproduction and human health. In the past, a network of genes, either germline-specific or non-specific, has been identified and characterized to be essential for male fertility based on high-throughput sequencing in the patient’s pedigree or GEMMs. However, it must be pointed out that, although the testicular structure and germline developmental process resemble each other to some extent between mice and humans, there are considerable differences in both species. For example, the meiosis persists for ~25 days in men but only ~14 days in male mice. Following meiosis, the haploid spermatid differentiation takes ~16 days in men but is shorter (~9 days) in mice. Thus, we must be cautious when interpreting the causative factors and mechanisms underlying human infertility by taking advantage of the findings from mouse studies [9]. In this review, we will particularly summarize those genes that are functionally important for post-meiotic male germ cell development in mice, which will aim in our understanding of human fertility.

2. Key phases during stepwise spermiogenesis

In mice, spermiogenesis entails the dramatic morphological transformation of round spermatids into hook-shaped mature spermatozoa. Unlike somatic cells, the motile sperm are capable of fertilizing an egg through their highly specialized cell structures [10]. Three major morphological changes take place in the process of spermiogenesis, including acrosome biogenesis, head reshaping, and development of a flagellum (Fig. 1). Right after meiosis, pairs of individual round spermatids are connected through intercellular bridges (Cytoplasmic bridges) through which haploid gene products transit and are shared across the cell membrane. On basis of the acrosome morphology, it can be divided into four phases during haploid spermatid development (Fig. 1) [11]. The first phase is called Golgi phase in which the proacrosomal vesicles fuse to form a single large acrosomal vacuole, which contains the granules in contact with the nuclear envelope (Step 1-3). Soon after meiosis, Golgi-derived vesicles traffic through microtubules and F-actin microfilaments, and subsequently aggregate and fuse to form the acrosome sac. Round spermatids consist of a centrosome with two centrioles - one is named distal centriole (mother centriole) based on the distal location while the other is called proximal centriole (daughter centriole). During sperm flagellar formation, the centrioles undergo dynamic remodeling and serve as the nucleators of the cilia axoneme, of which the process is explicitly reviewed elsewhere [12]. Upon occurrence of acrosomal sac, the centriolar pair migrates to the other pole opposite to the acrosomal vesicle wherein the axoneme starts to assemble. These distal centrioles will develop into flagellum while the proximal centrioles and pericentriolar matrix will eventually develop into head-tail coupling apparatus, which connects the sperm head and tail axoneme. In the second phase, also known as cap phase (Step 4-7), both the tail axoneme and acrosomal sac continue to grow. Meanwhile, the growing acrosomal sac becomes flattened and forms a cap tightly adhered to the anterior nuclear envelope through the F-actin-containing acroplaxome. The acrosome-acroplaxome complex descends caudally to the tail assembly direction in concert with the elongation of the nucleus. This process is followed by the acrosomal phase (phase 3), the acroplaxome attached to the acrosome continues to descend along the axoneme direction and spreads.
over the anterior nuclear envelope, in accompany with the developing of microtubule-containing ring-like manchette and the aligning of mitochondrion to the axoneme (Step 8-12). The outer dense fibers (ODFs) start to assemble and align along the developing axoneme. Upon completion of nuclear elongation, the acrosomes enter the maturation phase (phase 4) (Step 13-16) [13] when the manchette starts to dissemble and the majority of histones are step-wise replaced by protamines whereby the chromatin condensation continues further. This process necessitates genome-wide chromatin remodeling as well as massive single-stranded breaks (SSBs) and/or double-stranded breaks (DSBs) [14].

Currently, little is known about how the histones are globally substituted and how the SSBs/DSBs are repaired in such a narrow time window, although much knowledge has gained from GEMMs that the defect in any of these steps is closely linked to the compromised male fertility. In addition, it is noteworthy to point out that the acrosomal development unique to germ cells is successive and inherently connected to nuclear elongation and condensation. Secondly, since there is only one copy of allele owing to the haploid genome per se, spermatids are thus not capable of repairing the DSBs via homology-directed repair (HDR) mechanism. It is, therefore, conceivable that any disturbance at this stage would not only cause decreased fertility but also induce pathogenic mutations that can be carried over to progeny [15]. Finally, we must bear in mind that, although the sperm released into the lumen in the testicular tubules is morphologically identical to mature sperm, they must undergo further functional maturation during transit in the lengthy epididymis wherein the sperm might exchange information with extracellular vesicles in the fluid, in order to be capacitation-competent [16].

3. Morphological defects taking place during spermiogenesis

3.1. Acrosome assembly

As described above, the acrosome is a unique glycoprotein-rich organelle derived from Golgi complex, and thus can be readily visualized by the Periodic acid–Schiff (PAS) and PNA (Peanut agglutination assay) staining, or by electron microscopy [17]. The acrosome is also enriched in protease enzymes, such as acrosin, esterases, acid

Figure 1. Schematic diagram illustrating a total of 16 steps of haploid germline development in mice. Spermiogenesis is categorized into four phases (Golgi phase, Cap phase, Acrosome phase and Maturation phase) according to the acrosome morphology. Conventionally, spermatids are divided into 16 steps on basis of acrosome and head morphology, and the criteria is commonly leveraged to pinpoint the specific step of spermiogenic arrest through H&E staining of the sections from GEMMs. In literature, spermatids are often classified into four groups based on nuclear morphology: round spermatids (Steps 1-8), elongating/condensing spermatids (Steps 9-11), elongated/condensed spermatids (Step 12-14) and spermatozoa (Step 15-16). Representative genes essential for spermatid development are listed at the bottom.

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phosphatases, aryI sulfatases, and glycohydrolases, Sp56/AM67, SP-10, and Hyal-PH20, which are essential for sperm-oocyte fertilization. The acrosin and SP-10 are associated with the acrosomal matrix and released after the acrosomal reaction in large part [13]. SP56 is localized to the acrosomal matrix and has a specific affinity for mouse Zona pellucida sperm-binding protein 3 [18]. To date, Hyal-PH20 is the only ideal acrosomal membrane protein. It is a glycosylphosphatidylinositol-anchored protein and localized to both the inner acrosomal membrane and plasma membrane of sperm head. Interestingly, it has been shown to play in sperm-egg interaction that is unrelated to its enzymatic activity [19].

The sperm head shaping is an important event of spermiogenesis and is coordinated by nuclear condensation (Fig. 1). Two distinct subcellular structures are involved in the sculpting of the head into species-specific morphology (e.g., hook-like shape in mice, and oval shape in humans) -acroplaxome and manchette [20]. The anchoring of the acrosome to the nuclear surface is mediated via the acroplaxome, also named prenuclear theca, which is a thin layer of cytoskeletal elements between the nuclear and acrosomal membranes. In 2002, phospholipase C zeta [21] has been discovered, residing in the post-acrosomal sheath of perinuclear theca and is essential for oocyte activation [22]. The acroplaxome is comprised of myosin, F-actin and keratin-containing components essential for acrosome development. The acroplaxome provides mechanical support for transmitting forces around the anterior nucleus to facilitate the elongation of sperm head [23]. The presence of keratin-5 and F-actin is important in the acroplaxome for force generation and transfer [24]. On the other hand, it has been reported that some inhibitors related to microtubule dynamics interfere with the developing acrosomal vesicles resulting in abnormal spreading of acrosome over the nuclear membrane [25]. As a result, spermatozoa carrying defective acrosomes are not capable of penetrating zona pellucida of oocytes, leading to fertilization failure [26].

3.2. Manchette assembly

The second key structure that is essential for sperm head shaping is manchette, which is a transient skirt-like microtubular structure surrounding the posterior nucleus present in elongating spermatids (Fig. 1). It is composed of up to 1000 microtubules and consists of α and β tubulin heterodimers. The timing of manchette appearance is strictly step dependent. The short microtubules appear first around step 7 and then assemble at step 8 [27]. The microtubules are closely wrapping around posterior nuclear surface till step 14 when the manchette begins disassembling [28]. Through intra-manchette transport, it is thought to assist in the nucleocytoplasmic exchange, mechanical reshaping and elongation of sperm nucleus, as well as assembly of sperm tail. As a result, the excess cytosol is removed, and the nucleus becomes highly condensed. Any interference with the manchette structure or function can potentially give rise to the abnormal sperm head shape. Till now, a dozen of genes have been identified and are associated with manchette formation and development, which have been explicitly reviewed elsewhere [29]. Likewise, many mutagenic agents including inhibitors of microtubule dynamics, e.g., alkylating agent, can also induce manchette malfunction. Genetic deletion or mutation studies unveiled the aberrant manchette morphology, abnormal nuclear condensation/elongation and impaired fertility in those manchette-related gene mutation mouse models [30-32].

3.3. Flagellar assembly

The most distinctive feature of spermatids probably lies in the formation of a highly structured flagellum – the sperm tail, which is tightly relevant to sperm motility (Fig. 1 and 2) [10]. Morphologically, the flagellum is highly conserved in its ultrastructure and can be divided into four parts: i) neck, ii) midpiece, iii) principal piece and iv) end piece (Fig. 2). The neck is also known as connecting piece or head-tail coupling apparatus, which anchors the tail to the head tightly through the capitulum and the segmented column as magnified under transmission electronic microscopy (TEM). This ultrastructure has been elaborately discussed by several review papers elsewhere [30, 33]. The midpiece is comprised of mitochondria and 9 ODFs, which surround the 9+2 axoneme. Two ODFs are replaced in the principal piece by the longitudinal columns of the fibrous sheath (FS) connected by transverse ribs. The end piece contains only the axoneme surrounded by plasma membrane [34]. Upon completion of nuclear elongation and condensation, the fully developed sperm migrate close to the lumen of seminiferous tubules and are ready to be released into the lumen in a process called spermiation [10].

As the acrosome begins to form, the pair of centrioles move to the opposite pole of acrosome to initiate axoneme formation (Fig. 1). The axoneme arises from the centrioles and extends out into the cytoplasm [24]. The axoneme is comprised of characteristic “9+2” microtubules wherein a central pair of microtubules are wrapped by nine doublet microtubules. Notably, these microtubules are post-translationally modified including tyrosinated,
acylated, and polyglutamylated modification [30, 33]. The outer doublets contain dynein motor arms that generate forces to endow waveform motion of the flagellum. Defects in post-translational modifications of tubulins or perturbations in microtubular arrangement can impair axoneme assembly, which can be visualized by staining acetylated tubulin or by electron microscopy [30, 33]. At the last step, prior to fertilization, the sperm must acquire its fertilizing capability in a process known as capacitation during transit in the female reproductive tract. Capacitation is linked to hyperactive motility acquisition, characterized by an increase in the amplitude and asymmetry of the flagellar bending, and genetic mutations causing defective capacitation are linked to male infertility [35].

Until now, whereas it remains challenging to decipher which signaling pathways are involved in the axoneme assembly and elongation in vivo, growing studies have discovered a large number of genes in humans with the mechanistic and phenotypic studies further characterized in mouse models, showing they are tightly associated with axoneme formation. Most often, mutations or disruptions to those genes can induce a severe type of morphological abnormality commonly observed in the clinics - multiple morphological abnormalities in flagella (MMAF), leading to male sterility, which has been elegantly summarized and reviewed in details [36].

4. Spermiogenic defects: Insight view from mouse models

Post-meiotic spermatids develop clonally in many species ranging from fruit flies to humans and remain connected to one another via intercellular bridges. These bridges work as a channel for sharing protein products or even small subcellular organelles such as chromatid body (CB) and mitochondria [37]. In this way, the bridges enable haploid spermatids to be functionally diploid [38]. Exogenous interruption or genetic mutations often result in aberrant spermiogenesis, and consequently, lead to the reduction of sperm number or motility, or morphologically or functionally defective sperm. On basis of these varied abnormalities, the most common phenotypes with clinical outcome can be generally categorized into four groups as discussed below.

Figure 2. Schematic illustration of the ultrastructure of mature mouse sperm. Sperm flagellum is structurally divided into three parts - midpiece, principal piece, and end piece; each part comprises the core axoneme, which is composed of the canonical “9+2” arrangement of microtubules, in the center. The vertical arrow points to the #1 pair of microtubules while the horizontal dash line parallels the central pair of microtubules, in the cross mark. The mitochondrial sheath and the outer dense fibers (ODFs) wrap around the mid-piece and principal piece, respectively. The connection part between sperm head and tail is the neck, also termed head-tail coupling apparatus or connecting piece (right panel) [34, 150].

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4.1. Oligozoospermia

Oligozoospermia is a common type of human infertility, which refers to the low sperm count in the semen. According to the WHO 2010 criteria, the average sperm count is more than 60 million per ml, and a count of less than 15 million per ml is considered oligozoospermia [39]. It is further sub-categorized into three classes: mild oligozoospermia (10-15 million/ml), moderate oligozoospermia (5-10 million sperm/ml), severe oligozoospermia (< 5 million sperm/ml) [40]. In addition, it is now known that the disturbance in the post-meiotic spermatid development will likely cause aberrant spermatid apoptosis or premature spermatid sloughing off the seminiferous tubular epithelia, resulting in the reduction of spermatid number and hence impaired male fertility. This syndrome is more often connected to teratozoospermia and asthenozoospermia.

Until now, few genes are reported to be responsible for solely low sperm count. Fibroblast growth factors (FGFs) family contains 22 known members [41], which are involved in important cellular processes such as differentiation, migration, mitogenesis, and cell survival [42]. FGF action is mediated through high-affinity binding to tyrosine kinase receptors, which include FGF receptor 1 to FGF receptor 5 (FGFR1-FGFR5) [43, 44]. The role of Fgfr1 in male infertility and spermiogenesis has been assessed by dominant-negative transgenic mouse models, showing that Fgfr1 contributes to sperm production and function as the sperm daily-out has been examined low [45]. Recently, whole-exome sequencing (WES) efforts revealed a handful of genetic variants that are potentially linked to human oligozoospermia, such as HAUS7 [46], M1AP [47], MAGEB4 [48], RPL10L [49], and ZMYND15 [50] (Table 1).

4.2. Asthenozoospermia

Spermatozoa own highly specialized structures, which discriminate them from other somatic cells, especially the long tails, which are required for sperm motility. Defective microtubular assembly of sperm flagellum or functional suppression directly dampen sperm motility and thus cause reduced male fertility known as asthenozoospermia. It is one of the common types of male infertility in the clinics [51]. Mouse model studies have shown that the structural integrity of the flagella is of extreme importance for normal sperm motility, and defects in the axoneme assembly render poor sperm motility [52]. Not surprisingly, WES has identified a large number of genetic variants relevant to asthenozoospermia, while each variant is likely responsible for just a small fraction of patients, particularly including ADCY10 [53], CATSPER1-4 [54], EIF4G1 [55], GALNTL5 [56], NSUN7 [57], PLA2G6 [54], SPAG17 [58], and TRPC5 [59] (Table 1).

As aforementioned, the functional unit of flagella is the axoneme, which consists of structural microtubules and motor proteins that cooperate to produce waveforms and generate progressive motion in a synchronized and structured manner [60]. Inner dynein arms (IDA) and outer dynein arms (ODA) are ATPase complexes consisting of multiple motor proteins and reflect distinctive functions in flagellar motility. Dynein axonemal heavy chain family proteins (DNAH1, DNAH2, DNAH17), IQ motif containing G (IQCG), and tektin protein family are structural constituents of axoneme. In patients with asthenozoospermia from different ethnic backgrounds, common deleterious mutations have been found in the genes encoding inner dynein complex and nexin-dynein regulatory complex. Nevertheless, those null mouse models have immotile sperm elicited by disassembly of the axoneme and consequently, are unable to fertilize eggs [61-63]. Tektin is a family of filament-forming cytoskeleton proteins that comprise tubulin and dynein heterodimers in axoneme. It has been assumed that these constitutive proteins contribute to axonemal microtubular stability and structural complexity [64]. In humans, five tektins (TEKT1, 2, 3, 4, and 5) are present in the flagella of spermatozoa. Disruption to these proteins led to a low level of ATP and clumsy movement of flagella [64-67].

Capacitation is a cAMP-dependent bicarbonate-induced process by which sperm develop the capacity to fertilize eggs. A key enzyme in the PKA pathway is a soluble adenylyl cyclase (sAC) in the sperm tail encoded by the Adcy10. Adenylate cyclase uses ATP as a substrate to generate cAMP, further catalyzed by PKA to phosphorylate downstream proteins [68]. Male mice deficient in sAC are infertile owing to a complete lack of sperm motility [69]. Noticeably, homozygous frameshift variants of ADCY10 have been reported in asthenozoospermia patients [53]. Another core messenger during capacitation is Ca2+ [70], although the nature of the source responsible for the inflow of Ca2+ remains uncertain. CATSPER1-4, four cation channel-like proteins, have pore-lining residues forming alpha subunits and three auxiliary subunits, and their amino acid sequences imitate those of the conventional four-repeat voltage-gated Ca2+ channel with a single repeat. They are sperm-specific heterotetrameric Ca2+ selective channel proteins located on the middle and principal piece of flagella that regulate the calcium entry into the sperm, which is critical for the activation of sperm capacitation and motility. Mouse knockout models
exhibit drastically reduced sperm motility and are thus sterile only in the males. Recurrent genetic mutations were recently discovered in CATSPER family members in human patients with asthenozoospermia through Exome-sequencing, suggesting highly conservative roles of CATSPER members in sperm activation in mammals [54, 71-73].

Synchronized motion of the sperm tail involves energy production, commonly through oxidative phosphorylation, and signaling from its environment, and thus disturbances in the supply of energy and signaling transduction may also cause motility defects. It is generally speculated that oxidative phosphorylation is restricted to the midpiece harboring mitochondria, whilst glycolysis is confined to the principal piece [74, 75]. Several glycolysis isozymes are present in mammalian sperm, including Gapdh, Adcy10, and Vdac3. Glyceraldehyde 3-phosphate dehydrogenase-S (Gapds) is the only Gapdh isozyme tightly bound to the fibrous sheath. Voltage-dependent anion channel 3 (Vdac3) is a non-specific hollow porin in the outer mitochondrial membrane of sperm flagella. ATP levels significantly dropped in the Gapds- and Vdac3-null mice leading to non-progressive and weak motility of sperm flagella [69, 76-78]. However, for majority of patients with severe asthenozoospermia, the hereditary pathogenic variants, if present, remain largely undetermined, and further screening and functional studies are required to fully understand the pathogenesis of severe asthenozoospermia.

4.3. Teratozoospermia

Teratozoospermia is defined as a condition of male infertility characterized by the presence of abnormal morphology of sperm head or tail (Fig. 3), which is often linked to poor sperm motility. In patients with teratozoospermia, spermatozoa with irregular morphology account for more than 85% in the semen (Fig. 3). Teratozoospermia can be classified into polymorphic and monomorphic. Polymorphic teratozoospermia is thought to present an abundance of spermatozoa with heterogeneous forms of abnormalities. In contrast, monomorphic teratozoospermia exhibit a particular homogeneous morphological defect. Three well-described representative types of monomorphic teratozoospermia, i.e., macrozoospermia and globozoospermia, accephalic sperm syndrome have been reported thus far [26, 79, 80].

![Schematic representation of morphological abnormalities of sperm in mice and humans. A. Comparison of normal sperm head morphology between mouse and human. B. Common types of aberrant morphologies of sperm in mice and humans.](http://www.ijbs.com)
Nuclear elongation and chromatin condensation occur concomitantly, accompanied by the displacement of nuclear DNA-wrapped histones by protamines. Meanwhile, during this histone-to-protamine transition process, multiple histone variants are expressed, such as tH2A, tH2B, H1t, Hanp1, ssH2B, and Hils1, which are essential for sperm head maturation. Therefore, not surprisingly, genetic ablation of those histone variants usually caused severe defect in sperm condensation and male sterility [81-85]. In condensing spermatids, Tnp1 is loaded onto the nucleosomes, promoting protamines deposition and processing, and removal of histone proteins. Mice lacking Tnp1/2 are sterile, presenting poor motility and irregular chromatin condensation [86, 87]. Inside the sperm nucleus, the chromatin undergoes global modifications and remodeling during condensation, of which the process, however, remains largely unknown. We thus envision that further attempts to investigate the molecular changes throughout nuclear condensation will likely provide insights into the molecular mechanisms underlying patients with teratozoospermia.

4.3.1. Acephalic sperm syndrome

One rare subtype but highly severe condition is decaudated sperm (acephalic sperm or headless sperm). This syndrome is most often caused by the defective formation of the head-tail coupling apparatus (Fig. 2). Acephalic spermatozoa syndrome has been reported in humans for decades and is characterized by semen often containing sperm flagella without heads [88]. They are headless tails with a bulbous drop of residual cytoplasm but sometimes they are analyzed erroneously as a globozoospermia-like condition. A few acephalic spermatozoa-related genes, such as Sun5, Pmfbp1, Spata6, and Spatc1l, have been reported. Ablation of these genes in mice led to the production of acephalic sperm [89-92]. Sun5 has been identified to localize on the sperm nuclear membrane facing the acrosome and to bind the acrosome, and SUN5 mutations are most often found in patients suffering from acephalic sperm syndrome so far [93].

Teratozoospermia is commonly a severe consequence of defective flagellar assembly or destructive function of flagella. As discussed above, both intramantchette transport (IMT) and intraflagellar transport (IFT) are functionally similar to each other and both are important for the assembly and maintenance of cilia during the tail formation. By IMT, target cargo proteins are transported from nucleus to cytosol, of which the process is essential not only for nuclear condensation but also for tail assembly [94]. This is exemplified by protein-coding genes involved in cargo transport, such as Kif31, Meig1, Spef2 and Stk33. Deletion of Kif31 and Meig1 caused a substantial lethal effect on manchette formation and sperm tail production, resulting in deformed head (disrupted structure of manchette) and tail (axoneme disorganization, failure of flagellar formation) [95, 96]. Spef2 and Stk33 KO mice exhibited a similar phenotype with the irregular formation of manchette and ultimately highly disorganized tail structure [97, 98].

4.3.2. Macrozoospermia

Macrozoospermia is a very rare syndrome found in less than 1% of infertile males. It can be described as the presence of an enlarged sperm head resulting from the failure of nucleus condensation (Fig. 3). Currently, AURKC is the only well-known gene that manifests this kind of phenotype resulting in male infertility. Two brothers underwent genetic study of the AURKC gene presenting a relatively large-headed sperm phenotype. Aurkc deficient sperm of mice showed defects including heterogeneous chromatin condensation, and blunt heads with loose acrosomes [99, 100].

4.3.3. Globozoospermia

Globozoospermia is a rare type of teratozoospermia that accounts for less than 0.1% of male infertility. Any disruption to the acrosome formation might result in malformation of the acrosome and the condition is characterized by the presence of a round-shaped sperm head as well as the lack of acrosome [74]. The complexity of acrosome biogenesis, which lasts about two weeks in mice and one month in humans, makes it susceptible to endogenous or exogenous insults that may be detrimental for male fertility. Animal studies have unveiled a few genes and their pathways related to globozoospermia in haploid spermatids (Table 1), including Dpy19l2 [101], Dnah6 [102], Pick1 [103], and Spata16 [104], that account for the genetic causes of globozoospermia. In particular, DPY19L2 variants appear to be responsible for globozoospermia in most patients [105].

As described earlier, the fusion of Golgi-derived vesicles culminates in acrosome formation, of which the process has been tightly regulated and involves numerous vital players. Pick1 (protein interacting with C kinase 1) facilitates the vesicle trafficking from Golgi complex to acrosomes through protein trafficking. By gold particle labeling, it has been shown to localize in Golgi-related vesicles in round spermatids. Gmap210 is a Golgi membrane receptor for Ift20 and is critical for Golgi apparatus assembly [106, 107]. In these gene knockout mouse models,
ultrastructural analysis through TEM unveiled oblong head along with deformed acrosome, and abnormal formation of mitochondria sheath. Another gene, Hrb, encodes a protein related to nucleoporins essential for acrosome formation and deficient Hrb mice had round-headed spermatids and a complete lack of acrosome [108]. Dpy1912 (a transmembrane protein found in the inner nuclear membrane), Vps54 (Golgi-associated retrograde protein complex from the endosome for tethering of vesicles to the trans-Golgi network), and Mfsd14a (transmembrane protein) are responsible for the detachment of acroplaxome from the nucleus. Dpy1912 KO led to the intrusion of a layered structure present at the nuclear-acrosome junction, Vps54 deficient mice lack the true acrosome, and Mfsd14a-null mice failed to form an acrosome surround the nucleus [109-111].

Growing evidence showed that the autophagic molecular machinery, evolutionarily conserved from yeast to mammals, is involved in the transfer and fusion of Golgi-derived vesicles to the acrosome during acrosome biogenesis. The core members for autophagosome formation include ubiquitin-like conjugation proteins, Atg5/Atg7/Atg12 and microtubule-associated protein 1A/1B-light chain 3 (LC3) lipids. Atg7 is homologous to the ubiquitin-activating enzyme E1 essential for the conjugation structures [112, 113]. Atg7 mutant mice have aberrant round-headed spermatozoa with abnormal acrosome formation, which results from the failure of proacrosomal vesicle fusion and membrane trafficking [113, 114]. In round spermatids, Gopc partially colocalized with sp56 on the acrosome but failed to localize to the acrosome in spermatids deficient in Atg7 [115, 116]. Atg5 is also involved in the correct formation of acrosome, and upon knockout, Atg5-null sperm exhibit disorganized mitochondrion, misshapen heads and tails [114]. Interestingly, Sirt1 also appears to be involved in acrosome biogenesis via the autophagy pathway. The germline-specific Sirt1 KO mice have round-headed sperm with aberrant acrosome [117].

4.3.4. Multiple morphological abnormalities of the sperm flagella

The syndrome of multiple morphological abnormalities of the sperm flagella (MMAF) is considered as a specific subtype of asthenoteratozoospermia (Fig. 3) [52]. MMAF is characterized by mosaic anomalies of flagella involving coiled, twisted, irregular, shortened, or absent flagella [118, 119]. Other forms of asthenozoospermia, including MS defects, primary ciliary dyskinesia-related defects, ion channel defects and annulus instability should be distinguished from MMAF, and they are in general confined to the mid-piece that continuously displays mitochondrial ultrastructural changes or the lack of mitochondria [36]. Recent studies by WES screening have discovered a myriad of genetic variants as causes of MMAF patients, such as Dynemin axonemal heavy chain family proteins (DNAH1 [120], DNAH2 [121], DNAH6 [102], DNAH8 [122] and DNAH17 [123], and Cilia and flagella associated protein family (CFAP43 and CFAP44 [124], CFAP58 [125], CFAP65 [126], CFAP69 [127], CFAP70 [128], CFAP74 [63] and CFAP251 [129]). Apart from these two families, many other genes are also disclosed through WES, particularly AK7 [130], ARMC2 [131], DZIP1 [132], FSIP2 [133], QRICH2 [134], SPEF2 [135], TTC21A [136], TTC29 [137] and WDR66 [138] (Table 1). We expect that more other genetic variants concerning MMAF would be uncovered owing to the declining cost and advance of high throughput sequencing. The genetic mutations and featured phenotypes of mouse models for genetic variants discovered in MMAF patients have been explicitly discussed in several recent review articles [36, 139].

4.4. Oligoasthenoteratozoospermia

Oligoasthenoteratozoospermia (OAT) is the most common but severe form of pathogenic condition of male infertility characterized by a combination of semen abnormalities as described above, including low sperm count, poor motility, and abnormal morphology [140]. Noteworthily, OAT syndrome patients have sometimes been categorized into other types of defects in the clinics. Recent studies in both mouse models and human patients have revealed abundant genetic variants that account for OAT. However, the causative genetic mutations underlying at least 50% of OAT patients remain elusive. As mentioned above, any defect taking place throughout the prolonged development of haploid spermatids is likely to elicit OAT (see Table 1).

4.4.1. Intraflagellar transport (IFT)

As partially described above, numerous genes involved in intraflagellar transport relating to flagellar assembly exert vital roles during sperm head reshaping and tail formation. As a result, genetic mutations and functional disruption will cause OAT syndrome. Katnb1 and Lrguk-1 are predominantly expressed in germ cells and important for depolarization of microtubules during sperm head and tail axoneme development. Genetic inactivation of both genes resulted in OAT phenotype due to manchette dysfunction and tail defects [141, 142]. Ift20 and Ift140 are localized to the Golgi complex and involved in cargo protein transport among Golgi complex, microtubules and flagella, whereby the
excess residual cytosol is removed during flagellar assembly. There is no wonder that genetic disruption of Ift20 and Ift140 led to male infertility and OAT phenotype because of severe morphological defects in the sperm head and tails [143-145]. Likewise, IFT27 has been shown to bind IFT25 and is essential for flagellar formation even they are not required for ciliogenesis in somatic cells [146].

Table 1. Genes involved in spermiogenesis in mice and their corresponding mutation in humans.

| Gene Name | Knockout Mice Phenotype | Species | Phenotype | Reference(s) |
|-----------|-------------------------|---------|-----------|--------------|
| Bromodomain and WD repeat domain containing 1 (BRWD1) | Low sperm count, deformed spermatid heads, abnormal acrosome morphogenesis, defective chromatin condensation | Human | OAT | [151, 152] |
| Conctrator associated arginine methyltransferase 1 (Carm1) | Low sperm count, poor motility, abnormal morphology includes headless sperm with a bent midpiece | Mouse | OAT | [153] |
| Coiled-coil domain containing 62 (Ccd62) | Aberrant sperm phenotypes, impaired sperm motility, deformed acrosome, retention of cytoplasmic fragments on the sperm head and neck | Mouse | OAT | [154] |
| UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase3 (Galnt3) | Low sperm count, deformed acrosome, | Mouse | OAT | [149] |
| Germ cell less 1, spermaticogenesis associated (Gmcl/mGCL-1) | Low sperm count with poor motility, abnormal head, blunt acrosome, round/narrowed heads, insufficient chromatin condensation, multiple heads and flagella | Mouse | OAT | [155] |
| Homeodomain interacting protein kinase 4 (Hippk4) | Low sperm count, poor motility, aberrant head, abnormal tail (bent and coiled), ectopic localization of acrosome components | Human | OAT | [148] |
| Intrflagellar transport 20 (IFT20) | Low sperm count, poor motility, swollen head and abnormal tail (kinked tail, short tails or tailless) | Mouse | OAT | [145] |
| Intrflagellar Transport 140 (IFT140) | Low sperm count, poor motility, amorphous sperm heads, short/bent flagella | Human | OAT | [143, 144] |
| Katanin regulatory subunit B1 (KATNB1) | Low sperm count, poor motility, abnormal manchette, knob-like head | Human | OAT | [32, 156] |
| Leucine-rich repeats and guanylate kinase domain containing isoform 1 (Lgk-1) | Low sperm count, poor motility, misshapen heads, shortened tails, acrosome and the acroplaxome detached from the nuclear membrane in elongated spermatids, abnormal grass skirt-like manchette structure | Mouse | OAT | [141] |
| Makorin-2 (MKRN2) | Low sperm count, poor motility, aberrant morphology, spermiation failure, misarrangement of ectoplasmic specialization in testes | Human, Mouse | OAT | [157] |
| N-myristoylated-binding Protein (OSBP)-related Protein 4 (Orp4) | Abnormal head, distorted mitochondrial distribution | Mouse | OAT | [147] |
| Septin4 (Sept4) | Poor motility, abnormal morphology, defective annulus or bent neck, defects in mitochondrial architecture and acrosome morphology, sloughing of round spermatids, mitochondria with deformed acrosome | Mouse | OAT | [158] |
| Septin12 (SEPT12) | Low sperm count, poor motility, distinctive sperm morphology, deformed acrosome | Human, Mouse | OAT | [159, 160] |
| Serine protease inhibitor Kazal-type 2 (Snp2) | Missing proacrosomal vesicles fusion, disarrangement of the Golgi apparatus, and bent flagellum | Mouse | OAT | [161] |
| TATA-box binding protein associated factor 5 like (Taf5l) | Low sperm count, poor motility, abnormal morphology, compromised fertility | Mouse | OAT | [162] |
| Vacular protein sorting-associated protein (Vps13b) | Disorganization of the Golgi apparatus, degeneration of spermatid, deformed head | Mouse | OAT | [163] |
| HL7 linker histone (Hamp2/H1Q/H1fnt) | Poor motility, aberrant chromatin packaging, delayed nuclear condensation, detachment of acrosome | Mouse | OAT | [164] |
| Serine/threonine kinase 36 (Sk36) | Poor motility, club-shaped head with large gap between acrosome and the nucleus, absence of central pair of flagellar axonemes | Mouse | OAT | [31] |
| Adenylyl cyclase 10 (ADCY10) | Poor and lack of progressive sperm motility, deficit in cyclic adenosine monophosphate (cAMP) | Human | Asthenozoospermia | [53, 69] |
| A-kinase anchoring protein 4 (AKAP4) | Poor motility, diameter of principal piece reduced, distal flagellum splits apart into several filaments, incomplete development of fibrous sheath | Human | Asthenozoospermia | [165, 166] |
| Cation channel sperm associated 1-4 (CATSERPI-4) | Hyperactivated motility failure | Human | Asthenozoospermia | [54, 71-73, 167] |
| Polypeptide | Poor motility, lack of Acrosomal Component, ubiquitin-proteasome systems aberrant distribution | Human | Asthenozoospermia | [56, 168] |
| N-Acetylglalactosaminyltransferase Like 5 (GALNT5) | Poor motility, sluggish movement, bending of flagellar in the middle piece, declined concentration of ATP | Human | Asthenozoospermia | [76, 77] |
| Glyceraldehyde-3-phosphate dehydrogenase, spermaticogenic (GAPDH) | Poor motility, missing flagella, short and disorganized structure of tail, deformed nucleus | Mouse | Asthenozoospermia | [62, 169] |
| IQ motif containing G (IQCG) | Poor motility, lack of progressive movement and straight-line velocity, missing FS, longer ODF 1 and 9 with asymmetric mitochondrial line, Length of midpiece increased 2-3 times | Mouse | Asthenozoospermia | [120, 170] |
| Mouse dynamin heavy chain 7 (Dnmhc7/DNAPH1) | Poor motility, irregular nuclear shape with detached acrosomes, altered manchette microtubes, reduced chromatin condensation | Mouse | Asthenozoospermia | [58, 171] |
| Sperm Associated Antigen 17 (SPAG17) | Poor motility, defective flagellar pattern with abnormal bending between 90° and 180°, midpiece thinning | Human, Mouse | Asthenozoospermia | [64, 66] |
| Teckim-2 (TEKT2) | Bent flagella, loss of the inner dynein arm structures includes headless sperm with a bent midpiece | Human, Mouse | Asthenozoospermia | [67] |
| Teckim-3 Tekt3 | Poor motility, defective flagellar pattern with abnormal bending between 90° and 180°, midpiece thinning | Human, Mouse | Asthenozoospermia | [67] |
| Gene Name | Knockout Mice Phenotype | Species | Phenotype | Reference(s) |
|-----------|-------------------------|---------|-----------|--------------|
| Teckin-4 (Te4) | Poor motility, defect in flagellar bending restricted to the end piece, twitching motion of principal piece, disorganized sub-mitochondrial reticulum | Mouse | Asthenozoospermia | [65] |
| Transition protein 1 (TNP1) | Poor and lack of progressive motility, DNA breaks, abnormal pattern of chromatin condensation, less condensed chromatin, large rod-like structures | Human, Mouse | Asthenozoospermia | [86, 172, 173] |
| Voltage-dependent anion channel 3 (VDAC3) | Poor motility, loss of one outer doublet, axonemal defects, aberrant appearance of mitochondria along the midpiece | Human, Mouse | Asthenozoospermia | [78, 174] |
| Sperm associated antigen 6 (Spag6) | Low sperm count, poor motility, loss of the sperm head, midpiece fragmentation, truncated flagella, lack of central pair of microtubules, alterations in the outer dense fibers and fibrous sheath | Mouse | Asthenozoospermia | [175] |
| Aurora Kinase C (AURKC) | Defective nuclear chromatin condensation with defective acrosome, blunted heads | Mouse | Macrozoospermia | [99, 100] |
| ADP ribosylation factor like GTPase 3 (Arr3) | Abnormal head shape, lasso-like coiled tail | Mouse | Teratozoospermia | [176] |
| 5-aza-2′-deoxycytidine induced gene 1 (Azil1) | Defective manchette structure, misorientation of elongated spermatids, most of the sperm lack flagella while the remaining axoneme truncated | Mouse | Teratozoospermia | [177] |
| Cadherin 5 (CDH5) | Abnormal head morphology (nuclear deformation, partial failure of chromatin condensation) | Human, Mouse | Teratozoospermia | [178, 179] |
| CAP-GLY domain containing linker protein 170 (Clp-170) | Abnormal hook-shaped head, absence of manchette microtubules along with the nuclear envelope | Mouse | Teratozoospermia | [180] |
| Epithelial Microtubule-Associated Protein Of 115 (E-map115) | Abnormal head (Nuclei deformation, microtubules of manchette reduced) | Mouse | Teratozoospermia | [181] |
| Hypaectyulus (hd) | Detached centrosome, asymmetric and ectopic perinuclear ring of manchette, abnormal assembly of ODFs resulting in decapitated tails | Mouse | Teratozoospermia | [182] |
| Hook microtubule tethering protein 1 (HOOK1) | Elongated manchette with conically shaped, club-shaped nucleus, positional abnormalities of ODFs and FS result in lasso-like tail formation | Human, Mouse | Teratozoospermia | [183-184] |
| Kinesin family member 3A (Kif3a) | Low sperm count, malformed head, detached perinuclear ring, abnormal manchette, absence of axonemal and flagellar structures, displaced mitochondria, microtubules, ODFs, FS | Mouse | Teratozoospermia | [95] |
| Meiosis/Spermiogenesis Associated 1 (Meq1) | Disrupted microtubular organelle and flagellar formation, disrupted manchette | Mouse | Teratozoospermia | [96] |
| Polyamine Modulated Factor 1 Binding Protein 1 (PMFBP1) | Flagella without heads, retention of cytoplasm, abnormal axoneme assembly and misarranged mitochondria inside flagellum | Human, Mouse | Teratozoospermia | [90] |
| RIMS binding protein 3 (Rim-bp3) | Severe malformation of the sperm head and deformed nucleus with enlarged perinuclear space, misplaced acrosome | Mouse | Teratozoospermia | [185] |
| Spermatogenesis associated 6 (Spats6) | Accephalic spermatozoa | Mouse | Teratozoospermia (accephalic sperm) | [92] |
| spermatogenesis and centriole associated 1 like (Spats3) | Accephalic spermatozoa | Mouse | Teratozoospermia (accephalic sperm) | [91] |
| Sperm flagellin 2 (Spyf2) | Short and bent tail at all steps of spermatid elongation, absence/twin tails at steps 15-16, missing central pair of the axonemal microtubules and defective assembly of tail accessory structures | Mouse | Teratozoospermia | [98] |
| Spermatid maturation 1 (Spem1) | Poor motility, deformed head, completely bent tail, retained cytoplasm | Mouse | Teratozoospermia | [186] |
| Serine/threonine kinase 33 (Stk33) | Head-dislocation, Misarrangement of microtubules and mitochondria | Mouse | Teratozoospermia | [97] |
| Ubiquitin conjugating enzyme E2 B (UBE2B) | Aberrant head morphology, ectopic manchette, middle piece deformation | Human, Mouse | Teratozoospermia | [187, 188] |
| Armadillo Repeat Containing 2 (ARMC2) | Poor motility, short, coiled, and absent flagella, complete deficiency of motility | Human, Mouse | MFAF | [131] |
| Cilia and Flagella Associated Protein 43-44 (CFAP43-44) | Poor motility, disorganization in fibrous sheaths and periaxialomemal structures, distorted cytoskeletal components, lack of central microtubules with disorganized ODFs | Human, Mouse | MFAF | [124, 189] |
| Cilia and Flagella Associated Protein 65 (CFAP65) | Lack of progressive motility, absent or short flagella, lack of central microtubules with disorganized ODFs | Human, Mouse | MFAF | [126, 190] |
| Cilia and Flagella Associated Protein 69 (CFAP69) | Shortening of midpiece and principal piece, shorter flagella, abnormal axoneme morphology | Human, Mouse | MFAF | [127] |
| Dynactin Axonemal Heavy Chain 17 (DNAH17) | Impaired sperm flagellar assembly with disorganized axonemal structure | Human, Mouse | MFAF | [123] |
| Glutamine Rich 2 (QRICH2) | Sperm flagella with short, bent, coiled and other irregular shapes, displacement of the central microtubules and ODF, abnormal 9 + 2 structure | Human, Mouse | MFAF | [119, 134] |
| Tetra-tricopeptide Repeat Domain 21A (TTC21A) | Poor motility, aberrant structure of connecting piece, lack of central microtubules with disorganized ODFs, extra peripheral microtubule doublets, absent dynein arms | Mouse | MFAF | [136] |
| Tetra-tricopeptide Repeat Domain 29 (TTC29) | Poor motility (lower progressive motility), axonemal disorganization, lack of central microtubules with disorganized ODFs, | Human, Mouse | MFAF | [137] |
| Autophagy related 7 (Atg7) | Round-headed sperm, lack of acrosome | Mouse | Globozoospermia | [116] |
| Casein kinase 2 alpha 2 (Csnk2a2) | Abnormal head with bent flagella, deformed acrosome that detached from the nucleus | Mouse | Globozoospermia/Oligozoospermia | [191] |
| Dpy-19 like 2 (DFY39L2) | Round-headed sperm, midpiece defect, lack/deformed acrosome, 70% show absence of mitochondria while the | Human, Mouse | Globozoospermia | [105, 111] |
4.4.2. Acrosome formation

A handful of genes, such as Vps13b, GalNAc-T3, Orp4, and Hipk4, have been identified in the proper organization of Golgi apparatus and fusion of proacrosomal vesicles. Vps13b is involved in the sorting and transport of proacrosomal vesicles to the nuclear dense lamina in early spermatids. Oxyysterol-binding protein (OSBP)-related protein 4 (Orp4) is a cytosolic receptor with a high affinity for oxyesters and transport cholesterol among intracellular organelles. Galnt3 encodes UDP-GalNAc polypeptide N-acetylgalactosaminytransferase 3 that facilitates the fusion of proacrosomal vesicles. Hipk4 (homeodomain interacting protein kinase 4) functions as a critical regulator of the acrosome–acroplaxome complex. Genetic deletions of these genes all gave rise to OAT phenotype, mostly exhibiting fragmented and unfused acrosomes [147-149].

5. Prospects

Male germline development is a unique and sophisticated process in that it undergoes successive mitosis, meiosis and haploid differentiation. Abundant genes are spatiotemporally regulated and highly expressed in meiotic spermatocytes and round spermatids to guide correct spermatogenesis. As such, our current mechanistic understanding of male germline development is gained largely from mouse models. However, we must keep in mind that there are still limitations to using mouse models in that the germline in both species differs in many aspects, such as the different spermatogenic stem cell markers and differential chromatin condensation. In the clinics, while defective meiosis usually caused severe azoospermia, i.e., absence of sperm, any disturbance during the haploid stage most often caused either severe oligozoospermia, asthenozoospermia, teratozoospermia, or a combination (OAT). Luckily, the patients suffering from these kinds of syndromes can resolve to intracytoplasmic sperm injection (ICSI) approach, which is commonly adopted in the reproductive center and has been a powerful means to overcome fertility issues. However, we must bear in mind that the sperm in those patients with decreased fertility might be genetically defective, and thus in vitro assisted reproductive techniques might cause the defective DNA mutations to be carried over to the offspring. Additionally, due to the lack of the long-term health record of babies born through in vitro.
vitro fertilization (IVF) techniques, it is still not well-known or controversial if IVF techniques themselves, e.g., the culture medium, ROS exposure in culture, choice of sperm, etc, could somehow have an unexpected impact on progeny development in the future. Thus far, whole-genome screening by next-generation sequencing (e.g., exome-sequencing) combined with mouse models, have been validated as an effective way to investigate the causative roles and mechanisms of genetic variants of interest, and hence the landscape of genetic variants yet to-be-identified will undergo rapid expanding. This will lead to improved diagnosis and treatment for patients suffering sterility. In conjugation with the pre-implantation genetic testing (PGT), we envision that the transmission of more deleterious genetic mutations causing congenital diseases will be blocked from one generation to the next, further reducing the birth defects and improving human health worldwide.

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Author Contributions

M.A. drafted the initial manuscript. J.B. and W.Q. revised the manuscript with comments from S.A., I.U., J.C., L.W. and X.T.

Competing Interests

The authors have declared that no competing interest exists.

References

1. Lindsay TJ, Vitrikas KR. Evaluation and treatment of infertility. American family physician. 2015; 91: 308-14.
2. Fertilisation H, Authority E. facts and figures 2006: fertility problems and future. The Royal Society; 2010.
3. Punab M, Poolamets O, Paju P, Vihljajev V, Pomm K, Ladva R, et al. Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Human reproduction. 2017; 32: 18-31.
4. Cheng CY, Mruk DD. The biology of spermatogenesis: the past, present and future. The Royal Society; 2010.
5. Wacławska A, Kurpisz M. Key functional genes of spermatogenesis identified by microarray analysis. Systems biology in reproductive medicine. 2012; 58: 229-35.
6. Russell LD, Ettlin RA, Hikim APS, Clegg ED. Histological and histopathological evaluation of the testis. Wiley Online Library; 1993.
7. Cannarella R, Condorelli RA, Mongioi LM, La Vignera S, Calogero AE. Molecular Biology of Spermatogenesis: Novel Targets of Apparently Idiopathic Male Infertility. Int J Mol Sci. 2020; 21.
8. Sharma U. Paternal contributions to offspring health: role of sperm small RNAs in intergenerational transmission of epigenetic information. Frontiers in cell and developmental biology. 2019; 7: 215.
9. Muciaccia B, Boitani C, Berlaco BP, Nudo F, Spadetta G, Stefanini M, et al. Novel stage classification of human spermatogenesis based on acrosome development. Biology of reproduction. 2013; 89: 60-1,1-10.
10. Lehtiniemi T, Kotaja N. The Genetics of Postmeiotic Male Germ Cell Differentiation from Round Spermatids to Mature Sperm. Genetics of Human Infertility: Karger Publishers; 2017. p. 101-15.
11. Abou-Haila A, Tulsiani DR. Mammalian sperm acrosome: formation, contents, and function. Archives of biochemistry and biophysics. 2000; 379: 173-82.
12. Avidor-Reiss T, Carr A, Fishman EL. The sperm centrioles. Molecular and Cellular Endocrinology. 2020: 119987.
13. Khawar MB, Gao H, Li W. Mechanism of acrosome biogenesis in mammals. Frontiers in cell and developmental biology. 2019; 7: 195.
14. Champroux A, Torres-Carreira J, Gharagozloo P, Drevet J, Kocer A. Mammalian sperm nuclear organization: resiliencies and vulnerabilities. Basic and clinical andrology. 2016; 26: 1-22.
15. Cavé T, Desmarais R, Lacombe-Burgey C, Boissonneault G. Genetic Instability and Chromatin Remodeling in Spermatids. Genes. 2019; 10: 40.
16. Gervasi MG, Visconti PE. Molecular changes and signaling events occurring in spermatocoea during epididymal maturation. Andrology. 2017; 5: 204-18.
17. de Kretser DM, O’Donnell L. Phenotypic assessment of male fertility status in transgenic animal models. Spermatogenesis: Springer; 2013. p. 531-48.
18. Kim K-S, Cha MC, Gerton GL. Mouse sperm protein sp56 is a component of the acrosomal matrix. Biology of reproduction. 2001; 64: 36-43.
19. Ito C, Toshimori K. Acrosome markers of human sperm. Anatomical and Embryological. 2015; 170: 295-309.
20. Kierszenbaum AL, Tres LL. The acrosome-acroplaxome-manchette complex and the shaping of the spermatid head. Archives of histology and cytology. 2004; 67: 271-84.
21. Saunders CM, Larman MG, Parrington J, Cox LJ, Royle J, Blaney LM, et al. PLC zeta: a sperm-specific trigger of Ca2+ oscillations in eggs and embryo development. Development. 2002; 129: 3334-44.
22. Kashir J, Nomikos M, Swann K, Lai FA. PLCγ or PAWP: revisiting the putative mammalian sperm factor that triggers egg activation and embryogenesis. Mhr: Basic science of reproductive medicine. 2015; 21: 383-8.
23. Wong EW, Mruk DD, Lee WM, Cheng CY. Par3/Par6 polarity complex coordinates apical ectodermal specialization and blood-testis barrier restructuring during spermatogenesis. Proceedings of the National Academy of Sciences. 2008; 105: 9657-62.
24. Kierszenbaum AL, Tres LL, Rivkin E, Kang-Decker N, Van Deursen JM. The acrosomeplasm is the docking site of Golgi-derived myosin Va/Rab27a/b-containing proacrosomal vesicles in wild-type and Hrb mutant mouse spermatids. Biology of reproduction. 2004; 70: 1-10.
25. Huang W-P, Ho H-C. Role of microtubule-dependent membrane trafficking in acrosomal biogenesis. Cell and tissue research. 2006; 323: 495-503.
26. De Braekeleer M, Nguyen MH, Morel F, Perrin A. Genetic aspects of differentiation from Round Spermatids to Mature Sperm. Genetics of Reproduction and Andrology. 2014; 26: 110987.
27. Moreno RD, Schatten G. Microtubule configurations and post-translational α-tubulin modifications during mammalian spermatogenesis. Cell motility and the cytoskeleton. 2000; 46: 235-46.
28. O’Donnell L, O’Bryan MK. Microtubules and spermatogenesis. Seminars in cell & developmental biology: Elsevier; 2014. p. 45-54.
29. Pasch E, Link J, Beck C, Scheuerle S, Alsheimer M. The LINC complex and the shaping of the spermatid head. Archives of histology and cytology. 2004; 67: 271-84.
30. O’Donnell L, O’Bryan MK. Microtubules and spermatogenesis. Seminars in cell & developmental biology: Elsevier; 2014. p. 45-54.
31. O’Donnell L, Rhodes D, Smith SJ, Merriner DJ, Clark BJ, Borg C, et al. An essential role for katanin p80 and microtubule severing in male gamete production. PLoS genetics. 2012; 8.
32. O’Donnell L, Rhodes D, Smith SJ, Merriner DJ, Clark BJ, Borg C, et al. An essential role for katanin p80 and microtubule severing in male gamete production. PLoS genetics. 2012; 8.
33. Russell LD, Ettlin RA, Hikim APS, Clegg ED. Histological and histopathological evaluation of the testis. Wiley Online Library; 1993.
34. Lehti MS, Sironen A. Formation and function of sperm tail structures in association with sperm motility defects. Biology of reproduction. 2017; 97: 522-36.

http://www.ijbs.com
Hu TY, Zhang H, Meng LL, Yuan SM, Tu CF, Du J, et al. Novel
Okutman O, Muller J, Skory V, Garnier JM, Gaucherot A, Baert Y, et al. A
Curi S, Ariagno J, Chenlo P, Mendeluk G, Pugliese N, Sardi S, et al.
Cotton L, Gibbs GM, Sanchez-Partida LG, Morrison JR, de Kretser DM,
Padubidri V, Daftary S. Disorders of menstruation. Shaw’s Textbook of
Puga Molina LC, Luque GM, Balestrini PA, Marin-Briggler CI,
Morales CR, Wu XQ, Hecht NB. The DNA/RNA-binding protein,
Cooper TG, Noonan E, Von Eckardstein S, Auger J, Baker H, Brehm HM,
Tu C, Wang Y, Nie H, Meng L, Wang W, Li Y, et al. An M1AP
Greenbaum MP, Iwamori T, Buchold GM, Matzuk MM. Germ cell
intercellular bridges. Cold Spring Harbor perspectives in biology. 2011;
3: a005850.
Mallapragada R, Subramanian S, Upadhyay R, Roy A, Reddy R, Ojha P,
et al. A possible association of a human tektin-t gene mutation (A229V) with isolated non-syndromic asthenozoospermia: case report. Human reproduction. 2006; 23: 996-1001.
Roy A, Lin YN, Agro JE, DeMayo FJ, Matzuk MM. Absence of tektin 4 causes asthenozoospermia and subfertility in male mice. The FASEB Journal. 2007; 21: 1013-25.
Zuccarello D, Ferlin A, Carolla A, Pati MA, Moretti A, Cazzadorc et al. Possible association of a human tektin-t gene mutation (A229V) with isolated non-syndromic asthenozoospermia: case report. Human reproduction. 2006; 23: 996-1001.
84. Boissonneault G, Lau YF. A t-test-specific gene encoding a nuclear high-mobility-group box protein located in elongating spermatids. Mol Cell Biol. 1993; 13: 4323-30.
85. Moss SB, Challoner PB, Groudine M. Expression of a novel histone 2B duplication is required for spermatogonia development. Dev Biol. 1989; 133: 83-92.
86. Yu YE, Zhang Y, Unni E, Shirley CR, Deng JM, Russell LD, et al. Abnormal spermatogenesis and reduced fertility in transition nuclear protein 1-deficient mice. Proceedings of the National Academy of Sciences. 2000; 97: 4683-8.
87. Adham IM, Nayernia K, Burkhardt-Göttges E, Topaloglu Ö, Dixkens C, Dieterich K, Rifo RS, Faure AK, Hennebicq S, Amar BB, Zahi M, et al. Failure to assemble the peri-nuclear structures in GOPC deficient spermatids as found in round-headed spermatids. Archives of andrology. 2019; 1-15.
88. Perotti M, Gioria M. Fine structure and morphogenesis of ‘headless’ human spermatozoa associated with infertility. Cell biology international reports. 1981; 5: 113-.
89. Steng Y, Zhu F, Wang L, Ouyang Y-C, Dong M-Z, Liu C, et al. Essential role for SUN5 in anchoring sperm head to the tail. elite. 2017; 6: e28199.
90. Zhu F, Liu C, Wang F, Yang X, Zhang J, Wu H, et al. Mutations in PMFBPI cause aperinephric spermatogenesis syndrome. The American Journal of Human Genetics. 2018; 103: 188-99.
91. Kim J, Kwon JT, Jeong J, Kim J, Hong SH, Kim J, et al. Stk33 is required for spermatid differentiation and male fertility in mice. Journal of clinical investigation. 2009; 119: 802-12.
92. Yuan S, Stratton CJ, Bao J, Zheng H, Bhetwal BP, Yanagimachi R, et al. Abnormal spermatogenesis and reduced fertility in transition nuclear protein 1-deficient mice. Proceedings of the National Academy of Sciences. 2000; 97: 4683-8.
93. Adham IM, Nayernia K, Burkhardt-Göttges E, Topaloglu Ö, Dixkens C, Dieterich K, Rifo RS, Faure AK, Hennebicq S, Amar BB, Zahi M, et al. Failure to assemble the peri-nuclear structures in GOPC deficient spermatids as found in round-headed spermatids. Archives of andrology. 2019; 1-15.
94. Boissonneault G, Lau YF. A t-test-specific gene encoding a nuclear high-mobility-group box protein located in elongating spermatids. Mol Cell Biol. 1993; 13: 4323-30.
of the flagella but not to primary ciliary dyskinesia. Human Molecular Genetics. 2018; 27: 1196-211.

131. Coutton C, Martinez G, Kherraf Z-E, Arimi-Yekta A, Boguenet M, Saut A, et al. Bil-allelic mutations in ARM2 cause severe antherospermatogenesis due to sperm flagellum malformations in humans and mice. The American Journal of Human Genetics. 2019; 104: 331-40.

132. Lv M, Liu W, Chi W, Ni X, Wang J, Cheng H, et al. Homozygous mutations in DZIP1 can induce asthenotasterospermatogenesis with severe MFAF. Journal of Medical Genetics. 2020.

133. Martinez G, Kherraf Z-E, Zouari R, Fourati Ben Mustapha S, Saut A, et al. Whole exome sequencing identifies mutations in FIP12 as a recurrent cause of multiple morphological abnormalities of the sperm flagella. Human Reproduction. 2018; 33: 1973-84.

134. Kherraf Z-E, Arimi-Yekta A, Martinez G, Boguenet M, et al. Whole exome sequencing of men with multiple morphological abnormalities of the sperm flagella reveals novel homozygous QRICH2 mutations. Clinical genetics. 2019; 96: 394-401.

135. Liu W, Sha Y, Li M, Lin S, Huang X, et al. Loss-of-function mutations in SPEF2 cause multiple morphological abnormalities of the sperm flagella (MMAF). Journal of Medical Genetics. 2019; 56: 678-84.

136. Liu W, He X, Yang S, Zouari R, Wang J, Wu H, et al. Bil-allelic mutations in TTC21A induce asthenotasterospermatogenesis in humans and mice. The American Journal of Human Genetics. 2019; 104: 738-48.

137. Liu C, He X, Liu W, Yang S, Wang L, Li W, et al. Bil-allelic mutations in TTC29 cause male subfertility with asthenospermatogenesis in humans and mice. The American Journal of Human Genetics. 2019; 105: 1168-81.

138. Kherraf Z-E, Arimi-Yekta A, Dacheux D, Karaouzène T, Coutton C, Christou-Kent M, et al. A homozygous ancestral SVA-insertion-mediated deletion in WDR66 induces multiple morphological abnormalities of the sperm flagellum and male infertility. The American Journal of Human Genetics. 2018; 103: 400-12.

139. Jao S-Y, Yang Y-H, Chen S-R. Molecular genetics of infertility: loss-of-function mutations in humans and corresponding knockout/mutated mice. Human Reproduction Update. 2021; 27: 154-89.

140. Tüttelmann F, Ruckert C, Röpke A. Disorders of spermatogenesis. medizinische genetik. 2018; 30: 12-20.

141. Liu Y, DeBoer K, de Kretser DM, O’Donnell L, O’Connor AE, Merriner DJ, et al. LRGUK-1 is required for basal body and manchette function during spermatogenesis and male fertility. PLoS Genet. 2015; 11: e1005090.

142. Pleger C, Fietz D, Hartmann K, Weidner W, Kliessch S, O’Byrnck M, et al. Expression of katanin p80 in human spermatogenesis. Fertility and Sterility. 2016; 106: 1683-90.e1.

143. Wang X, Sha YW, Wang WT, Cui YQ, Chen J, Yan W, et al. Novel IFT140 variants cause spermatogenic dysfunction in humans. Molecular Genetics & Genomic Medicine. 2019; 7: e920.

144. Zhang Y, Liu H, Li W, Zhang Z, Zhang S, Teves ME, et al. Intrflagellar transport protein IFT140 (IFT140), a component of IFT-A complex, is essential for male fertility and spermiogenesis. Cytoskeleton. 2018; 75: 70-84.

145. Zhang Y, Liu H, Li W, Zhang Z, Zhang S, Teves ME, et al. Intrflagellar transport protein IFT20 is essential for male fertility and spermiogenesis in mice. Molecular biology of the cell. 2016; 27: 3705-16.

146. Zhang Y, Liu H, Li W, Zhang Z, Zhang S, Teves D, et al. Intrflagellar transport protein IFT27, an IFT25 binding partner, is essential for male fertility and spermiogenesis in mice. Developmental biology. 2017; 432: 125-39.

147. Charmian M, Colborne TR, Pietrangelo A, Kreplak L, Ridgway ND. Oxysterol-binding protein (OSBP)-related protein 4 (ORP4) is essential for cell proliferation and survival. Journal of Biological Chemistry. 2014; 289: 15705-17.

148. Crapster JA, Rack PG, Helinmann ZJ, Elias JE, Perrino JJ, Behr B, et al. HPK4 is essential for murine spermatogenesis. bioRxiv. 2019; 703637.

149. Miyazaki T, Mori M, Yoshida CA, Ito C, Yamatoya K, Moriishi T, et al. Whole-exome sequencing identifies mutations in KATNB 1 in the human testis and its genetic variants in fertile and oligospermatotaterospermatogenesis infertile men. Andrology. 2014; 2: 884-91.

150. Qian X, Wang L, Zheng B, Shi Z-M, Ge X, Jiang C-F, et al. Deficiency of Mkrn2 causes abnormal spermatogenesis and spermiation, and impairs male fertility. Scientific Reports. 2016; 6: 39318.

151. Kissel H, Georgescu M-M, Larisch S, Manova K, Hunnicutt GR, Steller H. The Sept4 septin locus is required for sperm terminal differentiation in mice. Developmental cell. 2005; 8: 353-64.

152. Lin Y-H, Lin Y-M, Wang Y-Y, Yu I-S, Lin Y-W, Wang Y-H, et al. The expression level of septin12 is critical for spermiogenesis. The American journal of pathology. 2009; 174: 1857-68.

153. Kuo YC, Lin YH, Chen HI, Wang YY, Chiou YW, Lin HH, et al. SEPT12 mutations cause male infertility with defective sperm annulus. Human reproduction. 2012; 33: 710-9.

154. Lee B, Park I, Jin S, Choi H, Kwon JT, Kim J, et al. Impaired spermatogenesis and fertility in mice carrying a mutation in the Spink2 gene expressed predominantly in testes. Journal of Biological Chemistry. 2011; 286: 29108-17.

155. Cheng Y, Buffone MG, Koudiao M, Goodheart M, Page DC, Gerton GL, et al. Abnormal sperm in mice lacking the Tafl1 gene. Molecular and Cellular Biology. 2007; 27: 2982-9.

156. Martinon J, Braconseri S, Catena R, Canssuller A, Kotaja N, Parvinen M, et al. Polar nuclear localization of H1T2, a histone H1 variant, required for spermatid elongation and DNA condensation during spermiogenesis. Proceedings of the National Academy of Sciences. 2005; 102: 2980-13.

157. Miki K, Willis WD, Brown PR, Goulding EH, Fulker KD, Eddy EM. Targeted disruption of the Akap4 gene causes defects in sperm flagellum and motility. Developmental biology. 2002; 248: 331-42.

158. Baccetti B, Collodel G, Estenoz M, Manca D, Moretti E, Plomboni P. Gene deletions in an infertile man with sperm fibrous sheath dysplasia. Human Reproduction. 2005; 20: 2790-4.

159. Avenarius MR, Hildebrand MS, Zhang Y, Meyer NC, Smith LL, Kahrizi K, et al. Human male infertility caused by mutations in the CATSPER1 channel protein. The American Journal of Human Genetics. 2009; 84: 505-10.

160. Takasaki N, Tachibana K, Ogasawara S, Matushika H, Hayjuda J, Ishizaka T, et al. Heterozygous mutation of GNLNT1 affects male infertility with impairment of sperm motility. Proceedings of the National Academy of Sciences. 2014; 111: 1120-5.

161. Harris TP, Schimenti KJ, Munroe RJ, Schimenti JC. IQ motif-containing G protein expressed predominantly in testes. Journal of Biological Chemistry. 2002; 277: 6298-305.

162. Cheng Y, Buffone MG, Koudiao M, Goodheart M, Page DC, Gerton GL, et al. Abnormal sperm in mice lacking the Tafl1 gene. Molecular and Cellular Biology. 2007; 27: 2982-9.

163. Meistrich ML, Mohapatra B, Shirley CR, Zhao M. Roles of transition nuclear proteins in spermiogenesis. Chromosoma. 2003; 111: 483-8.

164. Nourani T, Sumarsh T, Paramita R, Saleh MI, Narita V, Moeoek L, et al. Mutations in exons 5, 7 and 8 of the human voltage-dependent anion channel type 3 (VDAC3) gene in sperm with low motility. Andrologia. 2012; 44: 46-52.

165. Sapiro R, Kostetskii I, Olds-Clarke P, Gerton GL, Radice GL, Strauss III JF. Male infertility, impaired sperm motility, and hydrocephalus in mice deficient in sperm-associated antigen 6. Molecular and cellular biology. 2002; 22: 6298-305.
176. Qi Y, Jiang M, Yuan Y, Bi Y, Zheng B, Guo X, et al. ADP-ribosylation factor-like 3, a manchette-associated protein, is essential for mouse spermiogenesis. Molecular human reproduction. 2013; 19: 327-33.

177. Hall EA, Keighren M, Ford MJ, Davey T, Jarman AP, Smith LB, et al. Acute versus chronic loss of mammalian Axl/Cep131 results in distinct ciliary phenotypes. PLoS genetics. 2013; 9.

178. Zhuang T, Hess RA, Kolla V, Higashi M, Raabe TD, Brodeur GM. CHD5 is required for spermiogenesis and chromatin condensation. Mechanisms of development. 2014; 131: 35-46.

179. Peyser A, Bristow S, Puig O, Pollock A, Mills A, Niknazar M, et al. Do mutations in CHD5 cause male infertility? Fertility and Sterility. 2017; 108: e140-e1.

180. Akhmanova A, Mausset-Bonnefont A-L, van Cappellen W, Keijzer N, Chen H, Zhu Y, Zhu Z, Zhi E, Lu K, Wang X, et al. Detection of the azh/azh mutant mouse. Molecular reproduction and development. 2000; 104: 6852-7.

181. Komada M, McLean DJ, Griswold MD, Russell LD, Soriano P, Suryavathi V, Khattri A, Gopal K, Rani DS, Panneerdoss S, Zhou J, Du Y-R, Qin W-H, Hu Y-G, Huang Y-N, Bao L, et al. RIM-BP3 is a retinoic acid-inducible gene required for spermatogenesis. Genes & development. 2005; 19: 2501-15.

182. Mochida K, Tres LL, Kierszenbaum AL. Structural and biochemical features of fractionated spermatid manchettes and sperm axonemes of the azh/azh mutant mouse. Molecular reproduction and development. 1999; 52: 434-44.

183. Zheng H, Stratton CJ, Morozumi K, Jin J, Yanagimachi R, Yan W. Lack of Spem1 causes aberrant cytoplasm removal, sperm deformation, and male infertility. Proceedings of the National Academy of Sciences. 2007; 104: 6852-7.

184. Chen H, Zhu Y, Zhu Z, Zhi E, Lu K, Wang X, et al. Detection of heterozygous mutation in hook microtubule-tethering protein 1 in three patients with decapitated and decaudated spermatozoa syndrome. Journal of medical genetics. 2018; 55: 150-7.

185. Zhou J, Du Y-R, Qin W-H, Hu Y-G, Huang Y-N, Bao L, et al. RIM-BP3 is a manchette-associated protein essential for spermiogenesis. Development. 2009; 136: 373-82.

186. Liška F, Gosele C, Rivkin E, Tres L, Cardoso MC, Domaing P, et al. Rat hd mutation reveals an essential role of centrinob in spermatid head shaping and assembly of the head-tail coupling apparatus. Biology of reproduction. 2009; 81: 1196-205.

187. Escalier D, Bai XY, Silvius D, Xu PX, Xu X. Spermatid nuclear and sperm abnormalities of the sperm flagella in humans and mice. Journal of medical genetics. 2018; 55: 150-7.

188. Li W, Wu H, Li F, Tian S, Kherraf Z-E, Zhang J, et al. Novel variants in UBE2B gene and idiopathic male infertility. Journal of andrology. 2008; 29: 564-71.

189. Coutton C, Vargas AS, Amiri-Yekta A, Kherraf Z-E, Mustapha SFB, Le Tanno P, et al. Mutations in CFAP43 and CFAP44 cause male infertility with multiple morphological abnormalities of the sperm flagella in humans and mice. Journal of Medical Genetics. 2020; 57: 89-95.

190. Xu X, Toselli PA, Russell LD, Seldin DC. Globozoospermia in mice lacking the casein kinase II α’ catalytic subunit. Nature genetics. 1999; 23: 118-21.

191. Fujihara Y, Satouh Y, Inoue N, Isotani A, Ikawa M, Okabe M, Han F, Liu C, Zhang L, Chen M, Zhou Y, Qin Y, et al. Globozoospermia is required for spermiogenesis and chromatin condensation. Development. 2014; 131: 35-46.

192. Xu X, Toselli PA, Russell LD, Seldin DC. Globozoospermia in mice lacking the casein kinase II α’ catalytic subunit. Nature genetics. 1999; 23: 118-21.

193. Hall EA, Keighren M, Ford MJ, Davey T, Jarman AP, Smith LB, et al. Acute versus chronic loss of mammalian Axl/Cep131 results in distinct ciliary phenotypes. PLoS genetics. 2013; 9.

194. Audouard C, Christians E. Hsp90b1 knockout targeted to male germline: impact on spermatogenesis. Molecular human reproduction. 2013; 19: 327-33.

195. Liu G, Shi Q-W, Lu G-X. A newly discovered mutation in PICK1 in a patient with globozoospermia. Asian journal of andrology. 2010; 12: 556.

196. Hall EA, Keighren M, Ford MJ, Davey T, Jarman AP, Smith LB, et al. Acute versus chronic loss of mammalian Axl/Cep131 results in distinct ciliary phenotypes. PLoS genetics. 2013; 9.

197. Gao Q, Khan R, Yu C, Alsheimer M, Jiang X, Ma H, et al. The testis specific LINC component SUN3 is essential for sperm head shaping during mouse spermiogenesis. Journal of Biological Chemistry. 2020; jbc. RA119. 012375.

198. Li W, Wu H, Li F, Tian S, Kherraf Z-E, Zhang J, et al. Biallelic mutations in CHD5 cause male infertility? Fertility and Sterility. 2017; 108: e140-e1.

199. Pfister K, Perriat P, Wavin H, Gatt P, Corbeil E, Audouard C. Hsp90b1 knockout targeted to male germline: impact on spermatogenesis. Molecular human reproduction. 2013; 19: 327-33.

200. Lin Y-N, Roy A, Yan W, Burns KH, Matzuk MM. Loss of zona pellucida binding proteins in the acrosomal matrix disrupts acrosome biogenesis and sperm morphogenesis. Molecular and cellular biology. 2007; 27: 6794-805.