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Small RNA in spermatogenesis and male infertility

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1. ABSTRACT

Small RNA has become a crucial regulator of protein synthesis during spermatogenesis. Alterations in small RNA function prove to be detrimental to proper spermatogenesis. As many patients suffer from idiopathic infertility, understanding the molecular mechanisms of small RNA identifies possible causes of certain types of infertility. With a comprehensive review of the history of miRNA and piRNA function and specificity in the testis from a wide range of studies offers a view of detrimental defects of small RNA. By combining a concise overview of small RNA mechanism and recent research we explain how some cases of male infertility can be a product of complications in specific small RNA functions. The future direction section offers insight into how infertility treatment may be approached with a novel perspective.

2. INTRODUCTION

The process of spermatogenesis in mammals consists of three main phases. First, spermatogonia or germline stem cells are renewed via mitosis. Second, the primary spermatocytes undergo meiosis to produce round spermatids which are haploid. The third and final phase of spermatogenesis, spermiogenesis, is when the round spermatids mature into spermatozoa. The germ line stem cell undergoes mitosis two times forming spermatocytes. The spermatocytes undergo meiosis forming spermatids which eventually mature into spermatozoa with the support of Sertoli cells. Within each mitotic division and meiosis, a complex network of proteins is strictly regulated. Alterations at any of the above steps may result in hypospermatogenesis and male infertility. Among various causes of male infertility, genetic causes have explained maximum number of cases. However, 60-75% of male

infertility cases are still idiopathic (1), many of which display azoospermia and oligozoospermia. We must explore the molecular players in the process of spermatogenesis before we could understand the etiology of infertility in a larger fraction of infertile population.

One of the most remarkable processes during spermatogenesis is the drastic change in gene expression. It has been observed that as the differentiation proceeds, the genome gets packed tightly and is not available for transcription. As a result, transcripts must be stored in advance for possible use in the later stages of sperm differentiation, fertilization or further development (2). It has been recently proposed that small RNAs contribute a great deal of function in regulation of the gene expression in sperm (3). Many recent studies implicate small RNAs in the processes including: formation of the lumen during development, advancing spermatocytes through meiosis, assisting spermatid maturation and motility development (4). As the complex regulation of spermatogenesis is being unraveled, small RNA is becoming a prominent player in this process. As small RNA has a significant contribution in regulating the genome and transcription, variation in small RNA complexes could be detrimental to normal sperm development. Therefore, the implication of mutations/alterations in small RNA species must be considered in male infertility.

Small RNA was discovered in mammals about 9 years ago as micro RNA (5). Since then, several other small RNA species have been identified such as piwi interacting small RNA (piRNA) (6) which will be discussed as they have integral roles in spermatogenesis through multiple processes. The first known mechanism of action of small RNA was identified through miRNA and named RNA interference since its main function is to interfere with translation of mRNA, thus inhibiting the protein synthesis. The interference most commonly associated with small RNA is cleaving the mRNA transcript, but alternative methods such as attaching to the mRNA and occupying the accepting codon for ribosomal units or in the case of one function of piRNA, direct interactions with binding ribosomes in the polysome complex have been observed (7).

Changes in the expression of small RNA molecules could affect gene expression leading to compromise in the process of spermatogenesis. Recent studies have isolated clusters of small RNA and studied the expression patterns revealing specific processes directly affected by small RNA (8). Through many of these studies we can better understand how altered small RNA expression patterns may lead to male infertility. Looking at the increasing evidence of the role of small RNAs in spermatogenesis, we review the vast processes within spermatogenesis that small RNA is involved with, particularly miRNA, piRNA and their subspecies. With a thorough review of the available literature, we aim to understand why their function is necessary in spermatogenesis and how alterations in the production of these small RNA species might be linked to infertility.

3. miRNA, SPERMATOGENESIS AND MALE FERTILITY

miRNA is a ~22 nt single stranded small RNA first found to regulate gene expression by interfering with mRNA translation. This was first identified in *Caenorhabditis elegans* and later identified in several other organisms including humans 43 (3). Now, miRNA is believed to regulate expression of 60% of genes which encode proteins in the human genome. As of September of 2010, 1100 miRNAs have been sequenced on the human genome and that number is expected to grow rapidly in upcoming years (9). miRNA is involved in almost every biochemical process in the body through post transcriptional regulation (Table). Many of the miRNA templates are found in intron sequences and are transcribed into a double strand usually hairpin form pre-miRNA which must be cleaved by RNaseIII and unwound to form a single strand mature miRNA in the cytoplasm (10). This strand is unwound at the less stable 5' end and is loaded into a RNA-induced silencing complex (RISC.) RISC is composed of Argonaute proteins and most often interacts with the uncoding region of mRNA and has cleaving capability (3).

Other types of small RNA, specifically piRNA, also interact with RISC and specific Argonaute proteins (11) and control expression similarly to miRNA by binding to the complementary coding region of the mRNA and cleaving or suppressing expression by occupying the accepting region for translation of the mRNA. The code recognized by the small RNA does not always have to be identical which allows for some small RNAs to target multiple mRNAs and some mRNAs have many small RNAs capable of suppressing their translation. After cleavage, the miRNA remains intact and can pair with another mRNA strand. In 2007, Kedde *et al.* discovered that highly conserved protein Dead end (DND1) attaches to the 3' un-translated region of the mRNA, a familiar story to small RNA function (12). This discovery showed that DND1 occupies regions necessary for small RNA to attach, thus inhibiting small RNAs ability to silence. This process of small RNA regulation complicates the already tightly regulated process of small RNA posing another possible complication for proper small RNA function within spermatogenesis if DND1 levels are compromised.

One study sought out the profiling of testis specific miRNA on the mouse genome and found 141 miRNAs (13). Grouping patterns were observed, yet no definite pattern on the genome could be seen. Each chromosome contained miRNA coding genes except for the Y chromosome. The X chromosome showed significantly more miRNA coding genes than any other chromosome. One such cluster on the X chromosome, specific to primates, is expressed almost exclusively in the epididymis with predicted target proteins involved in morphogenesis of the epididymis, development of the lumen, regulation of immotile sperm indirectly and directly with flagella motility and maintenance of mature sperm. The protein SPAG6 (sperm-associated antigen 6,) which has been identified as an infertility protein, was also a predicted

Table 1. Some of the small RNA molecules important for male reproduction and fertility

Small RNA	Stage/site of Expression	Target Gene/Protein	Function of target gene/protein in spermatogenesis
miR-19a, miR-19b	---	PTEN	Tumor suppressant
miR-34b, miR-34c, miR-449	Primary Spermatocyte, elongating spermatid	NOTCH 1	Not expressed in patients with arrested spermatogenesis
hsa miR-154	Leydig Cells	AQP9	Water selective channels and secretion of tubule liquid
miR-122a	Round spermatocyte in chromatoid body	Tnp2	Assists in the replacement of histones
hsa-miR 181d	Pachytene spermatocytes and Sertoli cells	RNF6	Correlates with high transcription of maturing Sertoli cells
hsa-miR-218	---	CREB1, CASP2	Transcription factor interactions and apoptosis
miR 124a	During chromatin compaction	Sp3	Histone promoter
miR 449	---	BCl-2	Spermatogonial apoptosis
miR-883a, through miR3p	Late pachytene to spermatogonia	---	Suppresses X-chromosome
Nct1/2 (piRNA encoding sequence)	Heterochromatin	Transposable elements	Deficiency does not cause defect in spermatogenesis. Must be exclusively involved in transposon suppression
miR-17, through miR-92	Neonatal spermatogonia	---	Over expression may result in accelerated B cell lymphoma cell cycle while lower expression may result in progression into meiosis
AT-chX-1, AT-chX-2	---	Vas region located on the X chromosome	---
miR-372, miR-373	---	Oncogenic RAS and active wild-type p53	Apoptotic regulation and oncogenesis
Has-miR-890	Testis development	TEDDM1 (transmembrane epididymal protein 1)	Epididymal function and possible morphogenesis of the epididymis
Has-miR-888	Post meiotic division II	SPAG6,SPAG1	Sperm flagella motility and maintaining structure of mature sperm

target (4). These mechanism specific clusters are found throughout the genome, and especially on the X chromosome suggesting many stages of spermatogenesis that small RNA regulate at some level. Another study showed that many miRNAs derived from X chromosome templates are present in pachytene spermatocyte and the biogenesis of these miRNAs may escape meiotic sex chromosome inactivation (MSCI) (14). The study suggests that miRNA may be involved in suppressing the sex linked chromosomal transcription implicating small RNAs at all stages of spermatogenesis including maturation.

The grouping nature on the X chromosome and across the genome might imply that the miRNAs in clusters may target the same or similar mRNAs and perform similar functions or are involved in like processes for a broader spectrum of control by incorporating many targets within the same family of small regulatory RNAs. Also, when the pre-miRNAs are cleaved by RNaseIII, they go from large RNAs to many different types of miRNAs including sense and anti-sense forms. Often in other tissue types, one single strand from the original double stranded pre-miRNA is degraded but paired expression is observed in the testis which doubles the regulatory capacity of the target mRNA or similar mRNA molecules (15). Because towards the end of spermiogenesis, the chromosomes are tightly packed and transcription is limited, mRNA and miRNA must be synthesized before and stored for later use.

In mature sperm, about two-thirds of mRNA is partially stored in ribonucleoproteins in chromatoid bodies which are thought to be reservoirs for small RNA and mRNAs (16). The chromatoid bodies in post-meiotic germline cells in mice have been observed to be a storage location for Argonaute family proteins along with many other proteins and miRNAs and their target mRNAs (17). miRNA may act on mRNA within the chromatoid bodies. Some specific mRNAs are produced in meiotic cells and stored until translation is needed post-meiotically, most likely stored in the chromatoid body and regulated by miRNA. This discontinuous transcription implies an involvement in chromatin compaction, maintenance in the haploid cell and reconstruction which might be regulated by miRNA. Specifically, miR-122a in mice down regulates Tnp2 which is a protein involved in chromatin remodeling in spermatogenesis (16). Most transcription stops at the stage of elongating sperm since chromatin is tightly packed. The chormatoid body presence implies that miRNA is a prominent regulator both pre- and post-meiotically.

To best understand miRNA's involvement in spermatogenesis, researchers have knocked out the dicer gene for RNaseIII endonuclease in primordial germ line cells to observe how the absence of miRNA would affect spermatogenesis (18). It is important to note that this technique is not used to identify functions of specific clusters of small RNA, but generalizes the effect of a small RNA species deficiency. Knocking out the dicer gene should be used sparingly as more clusters are being identified. Small RNA profiling as a clinical tool can also be beneficial in identifying specific processes of small RNA clusters. However, knocking out the dicer gene displays major processes in which small RNA species may be integral. The initial developmental structure assessment of the seminiferous tubules in the Dicer knockout organism showed no difference to the control organisms. Retroposons suppression was also measured since miRNA

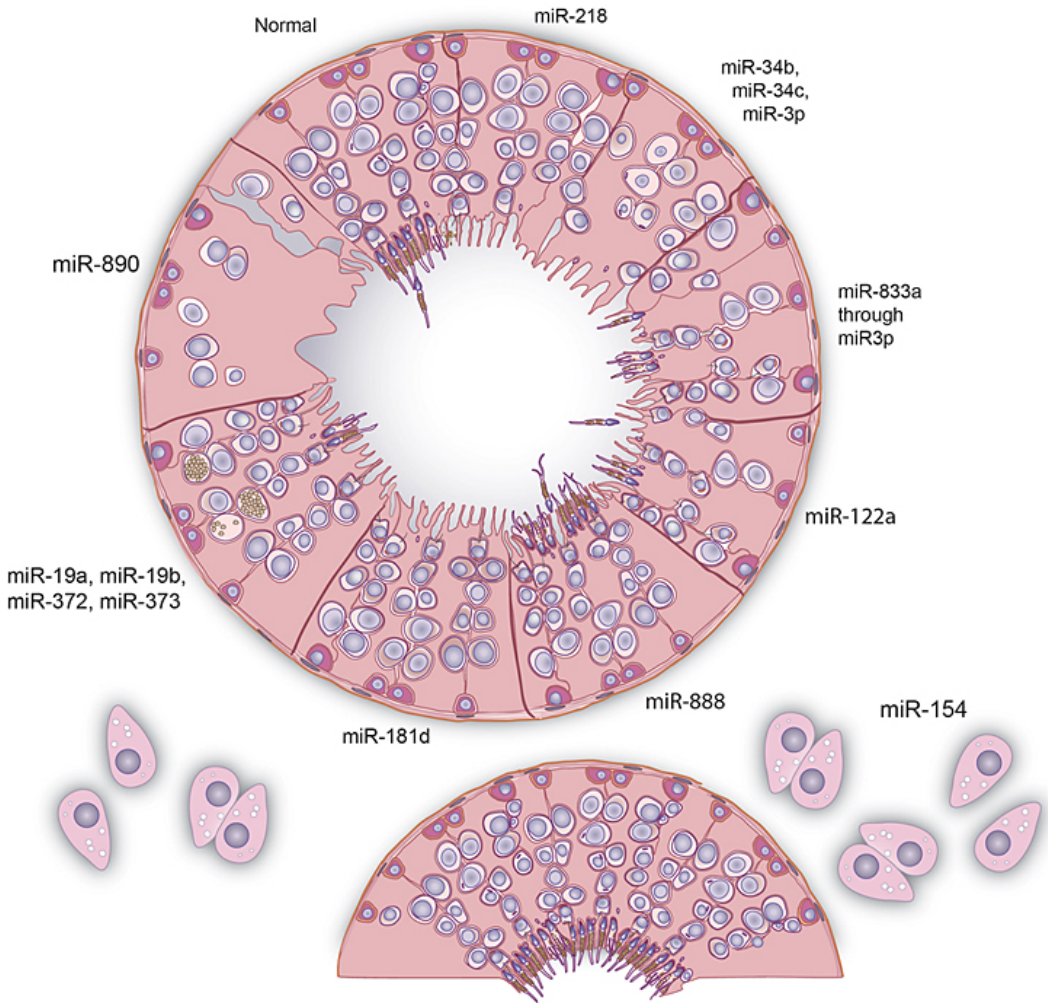

Figure 1. This diagram illustrates how a deficiency in the given small RNA might affect spermatogenesis phenotypically. Each portion or "slice" represents potential abnormality of the cells in the seminiferous tubules undergoing spermatogenesis. The diagram correlates with the Table appended in the article. The function of each small RNA mentioned in the figure is detailed in the Table. In order, beginning at the normal portion of the lumen and continuing clockwise, the illustration shows the probable phenotype due to deficiency of a particular small RNA. miR-218 is involved in the promotion of apoptosis, the lack of which might display an overgrowth in cells due to the lack of apoptosis. Next, miR-34b, miR-34c, miR-449 deficiency implicates as arrest in spermatogenesis at the stage of elongation with no fully formed sperm observed. miR-883 has been shown to be integral in the control of MCSI (meiotic sex chromosome inactivation.) A deficiency has shown to allow spermatogenesis to advance with few dysfunctional sperm with abnormalities resulting in infertility. miR-122 deficiency would result in histone replacement complications thus loose DNA packaging and increased apoptosis and reduced sperm production. Next, absence of miR-154 (presented outside of the main lumen) affects Leydig cell physiology and could affect proper testosterone production. miR-888 maintains sperm flagella and mature sperm morphology. An absence would cause abnormal mature sperm. miR-181d is usually expressed in Sertoli cells and spermatocytes and helps Sertoli cell development. Lack of this could result in less number of mature cells which could increase Sertoli cell proliferation resulting in an imbalance between the number of Sertoli and germ cells. miR-19a, miR-19b, miR-372, miR-373 are involved in tumor suppression, lack of which could result in tumor formation. Lastly, a lack of miR-890 would result in improper development of the lumen.

was thought to play a pivotal role in retroposon suppression in embryonic stem cells and a role in sperm retroposon suppression, but no difference was found between the control and experimental group. This can be explained by the importance of piRNA as the main source of retroposon suppression in germline cells. However, piRNA is not expressed in embryonic cells. This leads one to believe that the loss of miRNA might be compensated by another small RNA in regards to retroposon suppression.

Drastic level of spermatogenesis arrest was seen in Dicer knockout organisms at the early stages (18). It is important to note that apoptosis was not significantly increased in these knock out models but instead differentiation arrest was seen; whereas, in piRNA knock

out organisms apoptosis was significantly increased (19). Ago2 presence was also observed but no direct correlation to miRNA was noted. However, Ago2 is essential for small interfering RNA (siRNA) function which is an exogenously produced small RNA (20). High levels of Ago3 and Ago4 expression were observed in pachytene spermatocytes (21). The most apparent divergence was the lack of proliferation in spermatogonia suggesting that developing germ cells did not reach meiosis. The specific cluster of miRNA important in this proliferation has been identified as miR-17-92 (18). One possible process by which miRNA regulates cell proliferation is through the tumor suppressor protein PTEN which suppresses proliferation. Specifically, miR-19 a,b target the mRNA for this protein (18).

Not only has miRNA been identified as necessary for spermatogenesis in germline cells, but the knockout of the Dicer gene, the necessary RNase III for miRNA formation, in Sertoli cells of mice has shown to be detrimental to proper spermatogenesis (19). 248 miRNAs were identified in Sertoli cells, and 5 of them were Sertoli Cell specific (19). In the Dicer knockout Sertoli cells, these 5 miRNAs were completely suppressed. Because Sertoli cells are imperative in providing structural and nutritive support to germline cells and spermatogenesis, complete infertility was observed in these mice as no spermatozoa were found. The following were structural observations of these mutant mice highlighting the importance of small RNA in Sertoli Cell maturation: 80% decreased testis size, disorganized/absent lumen, misplaced Sertoli cells, and massive apoptosis of germline stem cells (19).

4. PIRNA, SPERMATOGENESIS AND MALE FERTILITY

piRNAs are small RNAs known to associate with argonaute proteins. Argonaute proteins function with piRNAs by guiding them to their target molecule for gene silencing (22). Argonaute proteins consist of two subclades, Ago and Piwi. These proteins can be further classified by their PAZ (Piwi Argonaut and Zwille) and Piwi domains. Murine models have demonstrated that three types of PIWI proteins and four Argo proteins exist: MIWI, MILI/PIWIL2, MIWI2/PIWIL4, and AGO1, AGO2, AGO3, AGO4. The MILI proteins are found in mice and rats up until the pachytene spermatocyte stage. The MIWI protein, however, can be found between the mid-pachytene and early round spermatid stages of spermatogenesis (22). A few major regions in the *Drosophila* genome have been identified as hotbeds of gene regulation. Both the *stellate* and *vas* regions of the genome are specifically targeted by the *Aubergine (Aub)* protein complex (23). *Aub* functions to silence retrotransposons in the germline (23,24) as well as the *stellate* region located in the testis (Table).

Drosophila males without Y chromosome are not only infertile, but also have abnormal spermatocytes with crystalline deposits in them (25). When a small region of the X chromosome, known as the *stellate* region, is deleted, it prevents the formation of these crystalline deposits. In *Aub* mutants, this is not possible, leading to increased amounts of transcript production from the *stellate region*. These transcripts code for the STE protein, a major component of the crystals found in abnormal spermatocytes Bozzetti *et al* 1995 (25). The *stellate* genes are silenced through the *Suppressor of Stellate* [Su(Ste)] repeats on the Y chromosome (26). When *Aub* is malfunctioning, it can lead to male infertility as *Aub* is unable to silence the repetitive sequences in the *stellate* region (23). Another important region targeted in Drosophila is the *vas* region. The two piRNAs associated with this region are called AT-chX-1 and AT-chX-2; both are derived from a small repetitive locus on chromosome X. *Vas* regions, encoding for factors involved in oocyte differentiation and cyst development (27,28), have been seen to be cleaved by *Aub*-piRNA complexes in the testes (23).

piRNA also targets the *flamenco* region in *Drosophila* (25). Believed to be a collection of defective transposons, the *flamenco* region is a heterochromatin structure located on chromosome X and one of the largest clusters of piRNA in *Drosophila* (29). It is primarily involved in down-regulating transposon activity through a process dependent on PIWI proteins. When the *flamenco* region is disturbed, it can lead to increased transposon activity due to decreased piRNA transcription (25,29). These possible disruptions in piRNA function have obvious consequences in increased amount of retrotransposons. Another result of piRNA mutations has showed increased amounts of retrotransposon repeats in the telomeres which compromise the protection function telomeres provide (11). The ping pong model is an example of how transposable elements misplaced in the genome can be regulated by the transposable element itself by the biogenesis of small RNAs. This self sustaining method has not been observed in humans, and no other method of self repair of small RNA deficiencies has been found in humans. However, existence of these mechanisms in other organisms such as *Drosophila* indicates possible existence of similar mechanism in humans too. The identification of such or a similar process could help understand possible mutations of the genome that could affect spermatogenesis and fertility.

The validity of mouse and rat experimental data of small RNAs applying to humans can be found in the conservation of certain piRNA clusters. 141 miRNAs were identified in the mouse genome testis expression in a study. Sequencing patterns were found to mirror certain piRNAs and mRNA (13). The grouping patterns of piRNA in the mouse, human and rat genomes were compared and showed syntanic large piRNA clusters (25). This homology not only verifies the credibility of some small RNA studies done in mice and other organisms as they correlate with humans but also implies conservation of certain small RNA molecules. Large clusters of piRNA have been found to be localized to similar locations within the rat genome and on specific human chromosomes (6). These clusters showing conservation across different classes of animals are piRNA clusters. Some clusters of miRNA involved in the regulation of spermatogenesis are products of more recent evolution. One study found a cluster of six miRNA specific to primates. Two of the six are expressed exclusively in the epididymis while the other four are abundantly expressed in

the testis and show low expression in other parts of the body (4). This suggests an evolutionary development of some miRNAs involved in the spermatogenesis process. In another testis specific primate study, 26 miRNAs which showed drastic expression patterns were shared by rhesus monkey and humans (30).

Along with similar location and function of piRNA clusters, a preference amongst mammalian piRNA for 5'U is observed (31). As far as *Drosophila* piRNA genomic patterns relate to humans, the main differences are more piRNA clusters localized to transposable elements and the ping-pong model specific to *Drosophila* and possibly other insect species (25). However, even though the ping-pong model has not been observed in humans, the transposable element suppression is a common theme amongst piRNA species in both humans and insects through different mechanisms. Conservation of piRNA clustering and functions between zebra fish, insects and humans makes piRNA conservation to be an obvious necessity for normal spermatogenesis. Apart from the conservation of retro-transposon suppression function, other piRNA epigenetic functions, such as chromatin structure and direct transcriptional repression have been proposed to be associated with PIWI proteins. Although some observations of epigenetic functions are exclusive to PIWI proteins, it is likely piRNA is involved in the protein complexes necessary for chromatin structure. One such report supporting piRNA function with PIWI protein in chromatin restructuring was observed when 3R-TAS1 piRNA mutant *Drosophila* had the 3R-TAS1 region located heterochromatically while usually it is semi-euchromatic (32).

piRNA also form a complex with MIWI in polysomes which would be another way to repress translation but not by directly interacting with the mRNA or transposon (7). The implication of a dysfunctional chromatoid body might be directly related to early embryonic abortion as the piRNA and miRNA loaded into the egg at fertilization from the chromatoid body is essential to protect the fertilized egg from transposable elements before the zygote can begin transcription on its own along with the small RNAs in the oocyte which play a crucial role in transposable element control (33).

5. SMALL RNAs AND MALE INFERTILITY

Although not all small RNAs discussed are identified in the human genome, the function is likely to correlate with a human counterpart targeting similar functional proteins. The current method of identifying certain small RNA species' targets is proposed through algorithmic testing in many programs such as: miRanda, PicTar and TargetScan (30). The relationship of the small RNA species to infertility can be precisely understood once the targets are identified and can encourage clinical attention to small RNA deficiencies in the genome.

In a study by Lian *et al.*, miRNA profiles of 3 patients with non-obstructive azoospermia were compared against 2 controls, and showed 173 miRNAs of at least a two-fold differentiated expression pattern, 154 of which were down-regulated (7.8% testis specific miRNA.) The study also noted a frequent down-regulation of mir-17-92 family in non-obstructive azoospermia patients (34). These alterations suggest the importance of appropriate levels of small RNA for fertility. But changes in small RNA levels are not the only contributor to infertility, the supporting proteins for small RNA must also be at proper levels to ensure normal spermatogenesis. For piRNA, mutations in the three types of PIWI proteins see normal development of the testes until 2 weeks post-partum, the time during which meiosis I is progressing. But after that period, post-meiotic cells are not seen (35). Specifically, the mutations in MILI/PIWIL2 and MIWI2/PIWIL4 proteins prevent spermatogenesis through the pachytene stage (25). Alterations in MIWI protein allow spermatocytes to become round-spermatids, but not mature spermatozoa (11). Beyond meiosis, miRNA has been identified as crucial to maintaining mature sperm and sperm motility (4). Small RNA profiling for specific cases of infertility could be beneficial to specifying the dysfunctional stage in spermatogenesis.

6. SMALL RNA BASED GENE THERAPY

The advancement of gene therapy has led to using synthesized small RNA molecules which associate well with RISC machinery in the cytoplasm and function the same way as naturally occurring small RNAs. Therapeutic RNA interference (RNAi) techniques seek to knockdown the expression of a gene by cleaving the mRNA. Synthesized small RNA molecules have been introduced as 20-30 nucleotide molecules called small interfering RNA (siRNA) and short hairpin molecules (shRNA,) similar in structure of pre-miRNA, which must be cleaved into siRNA. Most of the diseases to be treated with gene therapy require chronic RNAi. Because autoimmune responses to introducing endogenous DNA have caused serious complications in early gene therapy research, non-viral vehicles containing synthesized small interfering RNA has been a promising new technique (36).

The current complications involve uptake of the siRNA into the target cells, continual interference using realistic dosage and delivery of the siRNA and undesired silencing of genes not originally intended to be silenced (37). As we have seen, naturally small RNA molecules can have multiple targets and involve in the stringent control of many cellular processes which poses the most mysterious complication using siRNA treatment. However, many of these problems are being addressed rather successfully. One such clinical trial has used a nano particle complex consisting of cyclodextrin based polymer, targeting ligand, a hydrophilic polymer and the siRNA (38). The polymers are designed to protect the RNA from biological fluids, which would degrade the RNA, and also to conceal the negative charge of the RNA allowing for more successful transduction of the RNA (39). These alterations address the concerns of potency and uptake. The ligand is located on the edge of the vector and is used to associate with a specific target receptor on the target cell membrane

increasing the directness and aim of the treatment. This clinical trial was used to suppress the growth of a cancerous tumor, and the results were successful.

It is important however to note that each disease or deficiency being treated with gene therapy will have drastically different needs of potency, continual interference, and aim of the siRNA. For miRNA deficiency resulting in infertility or spermatogenesis arrest, the RNAi treatment may be less complicated. The current issue of undesired global silencing results from the introduction of an RNA species that may or may not have ever been present; meaning, the introduction of small interfering RNAs or synthesized small RNA molecules that do not mirror already existing anti-sense small RNAs designed to regulate the targeted gene. But with small RNA deficiencies discussed in this paper, it can be assumed that the siRNA introduced will in fact mirror a molecule that should be present normal. This should quell concerns of undesired effects of the knockdown of another non-targeted gene. Another chief concern is the duration of the RNAi. For most diseases, continual interference is necessary. But with the advancement of reproductive medicine technology, progressing spermatogenesis temporarily may allow retrieval of healthy sperm not seen prior due to arrest in spermatogenesis. If the small RNA deficiency can be identified, gene therapy treatment may not need to be given multiple times or in large quantities so long as spermatogenesis can be temporarily propelled. With exciting new methods of delivering the siRNA, certain spermatogenic complications may make some infertility patients, who have undergone genetic profiling, great candidates for future genetic therapy treatments.

7. CONCLUSION AND FUTURE DIRECTIONS

Through knockout gene mice and the use of siRNA, an exogenous small RNA, to silence certain gene, a number of small RNAs involved in spermatogenesis have been exposed. Recently, the number of studies exploring the transcripts in sperm has increased, and as a result the number of small RNAs identified is likely to increase soon. This would not only help understand the function of these small molecules, but also help understand alterations that could cause male infertility. By understanding the stages at which small RNA defects can be phenotypically observed, idiopathic infertility may be better explained. It appears that one of the major functions of small RNAs in the testis is to control the activity of transposons, which could be crucial for spermatogenesis. The ping pong model is an example of how transposable elements misplaced in the genome can be regulated by the transposable element itself by the biogenesis of small RNAs. This self sustaining method has not been observed in humans, and no other method of self repair of small RNA deficiencies has been found in humans. However, existence of these mechanisms in other organisms such as *Drosophila* indicates possible existence of similar mechanism in humans too. The identification of such or a similar process could help understand possible mutations of the genome that could affect spermatogenesis and fertility.

Abnormal nuclear packing of DNA and altered mRNA expression are thought to contribute to idiopathic infertility in males which can decrease IVF success. Small RNAs derived from novel oncogenes Mirn322 and Mirn323 are thought to mediate the expression of the mRNA derived from these genes and play a role in developing testicular germ cell tumors (GCT) (40). The expression profile of 156 miRNA differ in type II and type III for testicular cancer subjects suggesting importance of miRNA in male infertility due to testicular cancer in some cases (41). Because small RNA has been identified in intron sequences and heterochromatic sequences, the once thought to be silent mutations are becoming significant. 5-15% of infertile men or men exhibiting spermatogenesis problems have chromosomal abnormalities (42). Chromosomal aberrations can lead to genetic disorder such as Klinefelter's and Turner's syndromes but most of the time; the aberration will either restrict fertilization or cause very early abortion. Dicer knockout oocytes display meiotic I arrest spindle disorganization and high levels of transposons threatening the early embryo development. The wide array of functions of small RNA in spermatogenesis and even embryonic development warrant further studies on small RNA profiling of fertile and infertile men, which might allow for a better understanding to treat infertility.

miRNA can control transcriptional activity by methylation through epigenetic imprinting which should be studied in the future to reveal epigenetic patterns for embryonic development. Research in the last few decades has indicated strong role of epigenetic changes in regulation of the process of spermatogenesis and fertility. It has been observed that alterations in the epigenetic system could alter the function of important genes/ pathways in spermatogenesis/fertility. Therefore, studies in future could explore the influence of small RNA molecules on the process of epigenetic modifications which not only affects the individuals having the modifications but are also transmitted to the next generation. Therefore, the contribution and regulation exhibited by the small RNAs in epigenetic modifications could help further understand the process of spermatogenesis and associated infertility.

Infertility has been combated by assisted reproductive techniques (ART), especially in regards to extracting abnormally functioning sperm and fertilizing an oocyte via ICSI. Although ICSI allows sperm, normally incapable of reaching the oocyte or entering the oocyte, a chance to fertilize the egg, one must consider the genetic factors that may be compromised and are still being propagated to progeny through ART. Many sperm maturation steps have direct correlation with expression levels of certain small RNAs. Many of the specific functions of small RNA are not yet known, but the genes and proteins that they interact with are a window through which their involvement can be understood.

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