

Research Progress of Micro-RNA in Pathogenesis and Diagnosis of Male Infertility

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Abstract. 10% – 15% of couples worldwide experience infertility, with 50% of cases being attributed to male factors. Despite semen analysis being the most commonly used test to evaluate male fertility, it has some limitations. Consequently, reproductive scientists are exploring novel molecular markers that may aid in detecting sperm defects and facilitate improved diagnostic tools and biomarkers for male infertility. MicroRNAs, Over 60% of protein-coding genes are regulated post-transcriptionally by short non-coding RNAs, have potential as disease-specific markers when their expression is altered in body fluids. This article provides a systematic review of microRNAs' roles in different types of male infertility caused by sperm defects, including azoospermia, oligozoospermia, asthenozoospermia, and teratospermia, suggesting they may serve as new biomarkers to enhance diagnostic accuracy.

Keywords: biomarker, microRNAs, male infertility, azoospermia, oligozoospermia, asthenozoospermia, teratospermia.

1. Introduction

The failure of a couple to become pregnant after participating in regular, unprotected sexual activity for a year is known as infertility[1]. Male infertility is responsible for 50% of the cases of infertility, which affects up to 15% of couples worldwide[2-3]. Standard semen analysis is an important evaluation tool for the diagnosis of male infertility, but there are some restrictions, such as the inability to assess the potential of sperm to fertilize oocytes, their capacitation in the heterosexual reproduction system, and the difficulty in obtaining surface proteins for the assessment of sperm[4]. At the same time, the process from sample collection to result report in semen analysis is also error-prone, resulting in unreliable examination results. Electron microscopy and flow cytometry are two of the best methods for acrosome reaction detection. The bead binding assay (IBT) of antisperm antibody (ASA) can provide detailed and sensitive results, but these methods are expensive[5] Sperm function testing is an effective method to evaluate various parameters and the capacitation of sperm[6]. Although a large number of sperm function tests have been conducted, the reason for 40% of male infertility is still unclear[7]. Therefore, there is an urgent need to find new biomarkers that are specific, easy to quantify, and do not require invasive manipulation to assess male infertility. As an emerging biomarker, microRNA has stronger tissue specificity and shows different expression patterns in different organs[8-12]. In addition, microRNA was abundant in body fluid and produced earlier than protein. MicroRNA has the potential to be used as a diagnostic biomarker for male infertility, including conditions such as azoospermia, oligozoospermia, asthenozoospermia, and teratospermia.

Research has demonstrated that microRNA plays a significant role in regulating the entire process of spermatogenesis as well as in development and maturation. If microRNA regulation is dysfunctional, it will lead to various problems related to male infertility. New research has discovered that the level of modification of microRNA is associated with various diseases, especially male infertility[13-17]. More and more studies have found that weak sperm and normal spermatogenesis have different microRNA expression patterns. In 2013, the high-throughput microRNA microarray platform was used to detect the semen of patients with asthenospermia, oligoasthenospermia, and the normal group. The research demonstrated that in the

asthenospermia group, 50 microRNAs showed up-regulation while 27 microRNAs were down-regulated. Similarly, in the oligospermia group, 42 microRNAs were up-regulated and 44 microRNAs showed down-regulation. There were 34 commonly expressed microRNAs in the asthenospermia group and the oligoasthenospermia group, including up-regulation of expression of 27 microRNAs and down-regulation of expression of seven microRNAs[18]. Compared with the normal group, a 2016 study found that patients with oligozoospermia expressed 36 significantly different microRNAs, of which seven were up-regulated, with miR-1275 as the most significant, and 29 were down-regulated, with miR-26b as the most significant[19]. Research on Dicer1 knockout mice has suggested that the deletion of the Dicer gene may lead to a reduction in sperm size as well as an impairment in the ability of sperm cells to fertilize oocytes in vitro[20-21].

This paper provides a review of the regulatory role that microRNAs play in the development of male infertility as well as how this information can be applied for the diagnosis of male infertility through the use of bioinformatics.

2. microRNA family associated with male infertility

In recent years, the function of microRNA has been studied deeply, which affects the spermatogenesis process by regulating the transcription and translation of specific target genes[22]. RNA molecules have a significant role in controlling how genes are expressed in cells, with the majority of RNA being non-coding. MicroRNAs are one type of regulatory RNA that primarily acts post-transcriptionally by binding to specific target mRNAs, thereby influencing mRNA translation or stability. This regulation can ultimately impact protein expression and cellular processes[23-25]. MicroRNA is initially transcribed into primary microRNA (pri-microRNA) in DNA. After the generation of primary microRNAs, a complex composed of DROSHA and DGCR8 processes these molecules further into pre-microRNAs. The prerequisite microRNA was then transported from the nucleus to the cytoplasm by exportin-5. The microRNA gene in the intragenic and intergenic regions is transcribed by RNA polymerases II and III, producing a molecule shaped like a hairpin. Subsequently, these molecules were shortened to about 70 nucleotides by the RNase III enzyme DROSHA, becoming pre-microRNA[25 -29]. Exportin-5 transported the precursor to the cytoplasm, where it was cleaved into small, mature microRNA duplexes by the RNase III enzyme, again referred to as the Ago2/Dicer complex[30]. After that, the passenger chain is usually degraded, while the leader chain is assembled into an RNA-induced silencing complex (miRISC) to achieve the gene regulation of microRNA by binding to its targeted mRNA[31]. By binding to the 3'-UTR sites of target gene mRNAs, mature microRNAs are able to impede the translation or cleavage of the target mRNA[32]. Up until now, many microRNA families that are closely related to spermatogenesis have been found, including representative miR-34, miR-27, miR-146, miR-17-92, miR-202, miR-471, miR-29, and miR-151 families. The following is a detailed description of their roles:

2.1.miR-34 family

The MIRI-34 family is currently one of the more studied microRNA families. These microRNAs are specifically expressed in male germ cells and are closely related to meiosis. MiR-34b/c is the first microRNA site required for mammalian spermatogenesis, and its knockdown induces mouse infertility and abnormal sperm production[33]. MiR-34c can down-regulate its target gene, TGIF2, and thus promote the TGFB signal transduction pathway involved in the formation of pachytene spermatocytes and round sperm cells, thereby participating in spermatogenesis[34]. Through targeting the activated transcription factor 1 (ATF1) gene expressed in spermatocytes, miR-34c has been discovered to induce apoptosis in germ cells[35]. MiR-34c has also been found in high levels within spermatocytes and round sperm cells, and studies have shown that the inhibition of this microRNA can lead to a decrease in the rate of apoptosis in germ cells[36]. The reduction of PLCXD3 protein translation levels, which assists in the later stages of spermatogenesis, can be

regulated by miR-34c-3p. Studies have suggested that this regulation has been linked to potential occurrences of testicular dysfunction[37].

2.2.miR-27 family

The low or overexpression of miR-27b is related to the trend of low sperm motility and morphological abnormalities in patients with asthenospermia[38]. CRISP2 is an important protein for sperm motility[39].CRISP2 is commonly found in the dense fibers surrounding the acrosome and tail of sperm. During the acrosome reaction, CRISP2 is released from the acrosome to aid in regulating the movements of the sperm flagellum[40]. In patients with asthenospermia, the high expression of miR-27a mainly affects sperm morphology by inhibiting the same target gene, CRISP2, leading to the production of abnormal sperm[41].

2.3.miR-146 family

Research on mice has indicated that miR-146 exhibits increased expression levels in undifferentiated spermatogonia and is reportedly associated with the activity of the retinoic acid signaling pathway. This particular pathway plays a critical role in kickstarting the differentiation process for male germ cells and enabling their entry into meiosis[40]. Studies have revealed that increased levels of retinoic acid can impede miR-146 expression in undifferentiated spermatogonia. Conversely, an increase in miR-146 expression can counteract the impact of retinoic acid on spermatogonia[42].

2.4.miR-17-92 family

There is evidence to suggest that the miR-17-92 gene cluster may have a significant role to play in the initial differentiation of spermatogenic stem cells. This is due to its ability to decrease the expression of the E2F1 gene, which helps to prevent the onset of meiotic apoptosis in spermatogenesis[22]. Adult mice with the miR-17-92 gene cluster knockout exhibited reproductive dysfunction and an abnormal testicular phenotype, such as severe testicular atrophy, loss of spermatogonia and spermatogonial stem cells, and decreased germ cell apoptosis and spermatogenesis[43].

2.5.miR-202 family

According to research on miR-202, it was observed that the expression of miR-202 was significantly higher in spermatogenic stem cells of mice, and it displayed a reverse correlation with glial cell-derived neurotrophic factor (GDNF) and retinoic acid (RA), which together control the self-renewal and development of spermatogenic stem cells. In interstitial and supporting cells, increased levels of miR-202-5p were observed. In the absence of MIRI-202-3P, there was a notable acceleration in spermatogonial stem cell proliferation and an increase in spermatogonial cell apoptosis[44]. Glial cell-derived neurotrophic factor (GDNF) increased the expression of miR-202-3p, whereas retinoic acid was shown to down-regulate miR-202-3p and up-regulate the expression of miR-202-5p[44].

2.6.miR-471 family

A variety of microRNAs are found in Sertoli cells and Leydig cells, where testis-specific miR-471 may interfere with androgen activation of the Sertoli cells[45]. Furthermore, mir-471 is believed to modulate the functions of Dsc1 and Foxd1. DSc1 plays a critical role in maintaining the integrity of the blood-testis barrier, while Foxd1 plays a vital role in the maturation and development of germ cells following meiosis[45].

2.7.miR-29 family

The MIRI-29 family is also one of the microRNA families widely involved in the spermatogenesis process. It participates in transcriptional regulation before and during meiosis, repairs double-stranded DNA fragmentation, and regulates a variety of extracellular matrix components, thus playing a role in the dynamics of the basement membrane of germ cells[46].

2.8.miR-151 family

New research has indicated that the overexpression of miR-151a-5p in GC-2 cells can cause mitochondrial dysfunction, resulting in decreased basal respiratory oxygen consumption, ATP production, and proton leakage. These changes suggest that miR-151a-5p overexpression can impact the function of the mitochondrial electron transport chain and potentially harm sperm viability[22]. Furthermore, research has shown that miR-151a-5p is notably upregulated in the seminal plasma of individuals with asthenospermia. Conversely, miR-101-3p and let-7b-5p are significantly downregulated in these individuals[47].

2.9. other microRNA families

Research has identified miR-463 and miR-201 as genes with specific activity in the testis, potentially disrupting androgen activation in supporting cells[45]. The successful histone-PRM transformation required for advanced spermatogenesis cannot be achieved without the regulation of microRNA-mediated translation levels of transition proteins (TPs) and protamine (PRM), which involve the degradation process of testis-specific miR-469-targeted TP2 and PRM2 mRNA[47]. In addition, the disorder of miR-449 is also related to male infertility, which may lead to impaired sperm motility[49].

3. microRNA potential for diagnosis of male infertility

3.1 Azoospermia

Azoospermia, which is characterized by the total lack of sperm in the ejaculated semen, is brought on by several genetic flaws, such as chromosomal abnormalities, genetic abnormalities, and epigenetic flaws in the primordial germ cells[50-51]. Azoospermia is classified as obstructive azoospermia (OA) (stimulated by genital tract obstruction) or non-obstructive azoospermia (NOA) (stimulated by testicular abnormalities or deletions)[52]. To precisely categorize the sources of sperm abnormalities in the semen of men with azoospermia, there is currently no viable non-invasive diagnostic approach. In order to properly assess azoospermic males, a full medical history, physical examination, and hormonal profile are required. The workup and evaluation may be supplemented by imaging examinations, genetic testing, and testicular biopsy (with cryopreservation) [53]. Non-obstructive azoospermia is typically comprised of two histopathological patterns: pure supporting cell syndrome (SCOS) and spermatogenesis arrest defect (SA). The MirnEssy MicroKit (Qiagen) was used to separate and purify 20 seminal plasma samples in a recent study. The results demonstrate that men with lower miR-34c-5p transcript concentrations have lower sperm motility and normal morphology. Unaccounted-for infertile men were distinguished by the sperm miR-34c-5p transcript (AUC = 0.751, 95% CI: 0.568 - 0.934; p = 0.019)[54]. Furthermore, this change in miR-34c-5p expression was more pronounced in cases of pure supporting cell syndrome than in cases of defective spermatogenesis arrest[55]. In 2009, microarray-assessed expression of microRNA in testicular samples from non-obstructive azoospermia patients compared with fertile control samples identified for the first time 19 up-regulated and 154 down-regulated microRNAs[56]. Another study analyzed 77 samples and found significant changes in microRNA expression in patients with non-obstructive azoospermia. The study confirmed the upregulation of miR-539-5p, miR-370-3p, miR-22-5p, and miR-10b-3p, as well as the downregulation of miR-31-5p, miR-516b-5p, miR-34b-5p, and miR-122-5p[57]. In males with nonobstructive azoospermia and varicoceles, seminal plasma

miR-192a seems to be a promising marker for successfully signaling spermatozoa in the ejaculate after microsurgical varicocelectomy[58]. Additionally, microarray analysis identified 129 differentially expressed microRNAs in testicular tissue samples from patients with non-obstructive azoospermia, highlighting their role in spermatogenesis, cell cycle, and pre-mitosis[59]. Other research has demonstrated that the identification of specific microRNAs, such as hsa-miR-539-5p and hsa-miR-941, in extracellular vesicles may serve as a non-invasive diagnostic tool to predict the presence of sperm in patients with azoospermia prior to testicular biopsy[8]. Additionally, a diagnostic testicular biopsy appears to be of great value prior to varicocele repair in men with NOA and normal genetic testing[60]. Furthermore, research has linked the downregulation of hsa-miR-188-3p to the upregulation of MLH1 and the induction of sperm apoptosis, suggesting the potential of microRNAs as targeted therapeutic tools for azoospermia[61]. These studies indicate that microRNA has the potential to be a biomarker for the diagnosis of azoospermia and to develop targeted therapeutic tools[62].

3.2 Oligospermia

Oligozoospermia refers to the male ejaculation in which the sperm cell count is lower than the threshold of 15 million/ml, and it is divided into mild, moderate, and severe oligozoospermia[63]. Oligospermia is typically accompanied by morphological and sperm motility abnormalities[64]. Classification based on sperm density is limited because it does not define a specific threshold. For example, even men with sperm counts of 1 million to 5 million may become pregnant by natural means, and about 5% of fertile men also belong to different degrees of asthenospermia (oligospermia) type [65]. A microarray investigation of microRNA levels in men with oligoasthenospermia revealed the presence of 42 up-regulated and 44 down-regulated microRNAs, which target genes involved in spermatogenesis, apoptosis, sperm chromatin compaction, and sperm motility [18]. In another study, the expression levels of miR-34b-5p, miR-34b-3p, and miR-34c-3p were significantly reduced in oligozoospermia patients. Similar reductions in miR-34-5p, miR-34b, and miR-34c expression were observed in testicular tissues from patients with non-obstructive azoospermia[66]. Moreover, decreased expression of hsa-miR-34c-3p has been shown to inhibit the level of PLCXD3, which regulates cytoplasmic calcium, which is crucial to male fertility[67]. Therefore, hsa-miR-34c-3p and PLCXD3 are a significant microRNA-mRNA pair[68]. Additionally, miR-122-5p has a strong association with infertility and has great potential as a sperm quality biomarker [69]. Some researchers also hypothesize that oligozoospermia may be caused by miR-122-5p's low expression in semen samples [70]. Studies have shown that the abnormal change in miR-371a-3p expression level in semen is related to sperm concentration, and the reduced expression level may lead to oligospermia[71]. Estrogen is known to play a key role in sperm capacitation and fertilization potential[72]. In oligospermic patients, increased expression of hsa-mir-21 and hsa-mir-22 has been linked to reduced expression of estrogen receptors, demonstrating the regulatory effects of microRNAs on male infertility[73]. Moreover, a study analyzing microRNA levels in extracellular microvesicles from semen or testicular tissue samples, as well as seminal plasma in oligospermic patients, identified seven up-regulated and 29 down-regulated microRNAs potentially involved in spermatogenesis and its related processes [74].

3.3 Asthenospermia

Asthenospermia refers to the deficiency of male sperm motility, such as decreased progressive motility or a lack of motility [75]. The study by Wang et al. identified seven microRNAs that were up-regulated in patients with asthenospermia but down-regulated in patients with azoospermia, namely, HSA-MIRI-34C-5P, HSA-MIRI-122, hsa-iR-146b-5p, HSA-MIRI-181A, HSA-MIRI-374B, HSA-MIRI-509-5P, and HSA-MIRI-513A-5P[76]. Furthermore, low expression

of miR-374b is a possible non-invasive biomarker for the diagnosis of idiopathic infertile males[77]. Meanwhile, oligospermic patient sperm samples showed greater expression of miR-23a/b-3p, potentially due to the impact on their target genes, outer dense fiber 2 (ODF2) and ubiquitin 3 (UBQN3)[78]. According to one study, the expression level of hsa-miR-449-b in sperm samples of infertile men with oligozoospermia is down-regulated compared with that of normal men [79]. Compared with 30 normal men, semen analysis of 30 patients with asthenospermia showed a significant down-regulation of miR-525-3p and overexpression of Semenogelin-1(SE)G1 [78]. A study of 40 patients with asthenospermia and 40 men with normal sperm showed that miR-423-5p was overexpressed in patients with asthenospermia, which might be related to the inhibition of sperm movement by regulating cystatin S-transferase Mu 1 (GTSM1) [80]. CRISP2 is an important protein for sperm motility [39]. CRISP2, located in the acrosome and caudal part of sperm, regulates calcium influx through the ryanodine receptor, showing the regulatory role of microRNA in sperm movement [81]. It has been found that the expression of CRISP2 is low in semen samples with asthenospermia, while the expression of hsa-miR-27b is high [82]. Patients with asthenospermia showed a negative connection between miR-27a and CRISP2 protein expression [83]. Low sperm progression, aberrant morphology, and infertility were all substantially correlated with high miR-27b expression or low CRISP2 protein expression, respectively [84]. Semen samples from asthenospermic patients exhibited increases in hsa-miR-151a-5p and Samir-27B-3P, while hsa-miR-206 was down-regulated [85]. Semen samples from patients with oligo/asthenozoospermia and azoospermia were found to have lower levels of miR-135a, miR-10b, miR-135b, miR-891a, and miR-888 compared to healthy controls. [86]. Both miR-888 and miR-891a have a great capacity to recognize semen, although miR-891a has a higher discrimination accuracy [87]. In a study of 457 men of childbearing potential, seven microRNAs were significantly down-regulated in azoospermia patients, whereas miR-122, miR-146b-5p, miR-181a, miR-374b, miR-509-5p, and miR-513a-5p were up-regulated in those with asthenospermia [88]. Additionally, expression levels of miR-7-1-3p, let-7a, miR-141, miR-429, and miR-200a were up-regulated in sperm and seminal plasma samples from patients with asthenospermia and oligospermia, while miR-34b, miR-15b, and miR-122 were down-regulated, these miRNAs may be utilized as non-invasive indicators to identify men who have sperm production issues. [89]. Sperm motility is maintained by mitochondria, which are engaged in a number of biological processes [90]. MiR-151a-5p mimics were transfected, and Zhou and colleagues found that this lowered mitochondrial respiratory activity and the amount of adenosine triphosphate (ATP) [91]. Findings from research imply that miR-151a-5p may control cellular respiration and ATP generation by targeting cytochrome b. [92]. Another study revealed reduced levels of miR-4485-3p in semen samples from patients with asthenospermia compared to healthy individuals, however, Let-7b-5p has been demonstrated to affect glycolysis metabolism by concentrating on Aurora Kinase B (AURKB) [93]. Low expression of LET-7B-5P in severe ASZ has been reported when compared to the control group [94]. According to a bioinformatics analysis, these microRNAs were observed to play a significant functional role in regulating pathways associated with sperm motility [94].

3.4 Malformation of sperm

Teratospermia is a condition in which semen contains over 85% of sperm cells with abnormal morphology [95]. There are two subtypes of teratospermia: haplotype and polymorphic type, with monomorphic deformity indicating all sperm cells have the same abnormality and polymorphism indicating different types of abnormalities [96]. Malformed spermatozoa haplotypes are classified into macrospermia (abnormally large head) and coccidiosis (oval head) [97]. Aneuploidy, sperm DNA fragmentation, and mutations are only a few examples of the genetic causes that can cause sperm cells to develop abnormally from a morphological perspective [98]. Patients with teratospermia usually exhibit lower levels of miR-510-5p and higher levels of hsa-miR-328-3p and hsa-miR-296-5p in their semen samples [99],[100]. A study found that in 67 infertility cases,

miR-582-5p expression was elevated in the seminal plasma of teratospermia patients, while the expression of this microRNA in patients with teratospermia was decreased. The authors believed that miR-582-5p acts on CRISP2 and that this gene expression may lead to male infertility [100]. Corral-Vazquez et al. reported changes in expression of hsa-miR-942-5p/hsa-miR-1208 in cases of asthenospermia, hsa-miR-296-5p/hsa-miR-328-3p in cases of teratospermia, and hsa-miR-139-5p/hsa-miR-1260a in cases of oligospermia compared to control samples [99]. In another study, which included 71 infertile men, miR-510-5p expression was found to be reduced in semen samples from individuals with asthenozoospermia and teratospermia when compared to the normal sperm group [101]. Salas-Huetos et al. detected 32 microRNAs in samples from patients with asthenospermia, 19 microRNAs in samples from patients with teratospermia, and 18 microRNAs with altered expression levels in the oligospermia group. These microRNAs are believed to be involved in regulating normal sperm function[102].

4. Conclusions

Male infertility is a significant problem worldwide, and a significant number of patients are diagnosed with unexplained infertility despite the availability of various technologies to identify underlying causes. MicroRNA has recently emerged as a diagnostic biomarker with notable advantages throughout different stages of sperm production and maturation, facilitating the diagnosis of male infertility.

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