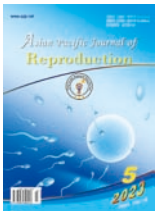

Review Article

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The role of small non−coding RNAs (sncRNAs) in male infertility: A scoping review

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ABSTRACT

Objective: To give a brief overview of the field of epigenetics and the potential predictive power that small non-coding RNA (sncRNA) may hold in relation to improving the treatment and diagnosis of male infertility.

Methods: PRISMA-ScR was used as the scoping review guideline for this investigation. All article data here have been accessed from MEDLINE–PubMed, Science Direct, EBSCO, Scopus, Sage Journals, and Google Scholar. The terms "small non coding RNA, male, infertility, miRNA, sperm" were used in the search between 2015 and 2023.

Results: The study comprised 35 publications in total. Several sncRNAs, miR-155, miR-16, miR-196, miR-525-3p, miR-891 were found to be effective in regulating the mechanism of spermatozoa processing in the infertility of men. sncRNA can be used as a biomarker of male infertility.

Conclusions: sncRNAs can act as biomarkers for the diagnosis of reproductive diseases. Actually, by recognizing sncRNAs and their mechanisms, a new way to treat infertile men would be paved. The functional annotation of sncRNAs in spermatogenesis is still in its infancy but has enormous potential. This is despite the fact that many potential sncRNAs have been found to date with the use of cutting-edge technology and publicly accessible sncRNA annotation tools.

KEYWORDS: Male infertility; miRNA; Small untranslated RNA; sncRNAs; Sperm; Next-generation sequencing; Real-time PCR

1. Introduction

The World Health Organization defines infertility as a couple's inability to conceive after a year of routine, unprotected sexual activity[1]. Fertility problems can affect either the male or the female, or both at times.

Low sperm numbers, poor sperm quality, or both contribute to more than 90% of male infertility cases[2]. Anatomical issues, hormone abnormalities, and genetic anomalies are among the other causes. According to a study, 30% of those who are deemed infertile are affected just by male factors, 20% by both male and female factors, and 15% are deemed infertile for unknown reasons[3]. Environmental, nutritional, medicinal, genetic, and physiological variables may all have a role in infertility in males[4]. As a result, there is still much to learn, and we will need to combine multiple different techniques to fully comprehend the etiology of male infertility.

Epigenetic mechanisms are one of many ways to understand the etiology of infertility, but the direct relationships between epigenetic changes and infertility have yet to be fully disclosed[5]. The important epigenetic mechanisms are DNA methylation, histone modifications, and small non-coding RNAs (sncRNAs) and they regulate male fertility from spermatogenesis to embryonic development[6].

SncRNA can also be seen as essential epigenetic modifiers, and if there is epigenetic dysregulation, it affects male infertility[7]. Sorts of non-coding RNAs (ncRNAs) consist of miRNAs (20–30 nt), medium ncRNAs (50–200 nt), and long non-coding RNAs (lncRNAs), which are longer than 200 nt[8]. SncRNAs have various activities in RNA modification, RNA interference, or spliceosome to the regulation of gene expression[9]. Post-transcriptional gene silencing (PTGS) and chromatin-dependent gene silencing (CDGS) are two mechanisms by which ncRNA molecules can inhibit the activity of target genes in the cytoplasm and nucleus[10].

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Since it may bind to target genes as either an imperfect or perfect complement, a given short RNA sequence can control the expression of several genes. Small RNAs are therefore just as crucial for biological activity as transcription factors, and they control more than 30% of the genes in a cell directly[11]. Short RNAs are crucial for biological functions such as cell differentiation, growth and proliferation, migration, apoptosis and death, metabolism, and defense. As a result, short RNAs play a crucial role in regulating healthy physiology and development[12].

Recent studies have indicated that sncRNAs are all required for spermatogenesis. They are crucial for spermatogenesis, and their dysregulation might cause male infertility. Many studies have shown that sncRNAs can be potential biomarkers of male infertility by regulating spermatogenesis[13].

Male germ cell formation, function, and role are regulated by siRNA, miRNA, and piRNA gene expression levels. In spermatogenesis, sperm piRNA and microRNA play an essential role and cause male fertility disorders. Piwi-interacting RNAs (piRNAs) are tiny in somatic cells, but they are numerous and contain the majority of the short RNAs in male germ cells. Male germ cells express miRNA at high levels in differentiated and undifferentiated conditions. In the formation of functional spermatozoa, whether miRNAs cooperate with other epigenetic modifications are regulated, and it is concluded that miRNAs are required at every stage of male germ cell development[14].

In recent years, the subclass of sperm sncRNAs involved in male infertility has been studied, and studies in this area have received greater attention. However, studies on the structure of sncRNAs in male infertility are quite limited. There is an extremely important correlation between sperm quality and sncRNAs. They are used as biomarkers useful in solving sperm-related problems[15]. Changes in the expression of some genes are directly related to sncRNA biogenesis' failure. In recent years, certain male infertility cases have been linked to dysregulation of miRNAs in sperm development, which could be used as biomarkers to diagnose the disease.

In this review, we attempted to summarize the effects of sncRNA molecules that play a role in male infertility based on the studies performed in recent years.

2. Methods

2.1. Protocol

Based on a methodology using the PRISMAScR checklist, this scoping review was carried out. The purpose of this scoping assessment was to determine the effects of sncRNA molecules that play a role in male infertility based on the studies performed in recent years.

2.2. Eligibility criteria

Selected publications did not include reviews, cancer, animal or plant articles. This article avoided reiterating the prior literature review publications regarding this field of study in order to discover the most recent papers. Articles were excluded if they were not written in English. The study included clinical studies on men with infertility and the findings of these studies. This article avoided reiterating the prior literature review publications and cancer, animal and plant articles. Manual removal of duplicate publications was done.

2.3. Information search

PRISMA-ScR was used as the scoping review guideline for this investigation. All article data here have been accessed from MEDLINE–PubMed, Science Direct, EBSCO, Scopus, Sage Journals, and Google Scholar.

2.4. Searching evidence

The search terms ‘small non coding RNA, male, infertility, miRNA, sperm’ were used. In certain publications, the expression of sncRNAs was evaluated using high-throughput sequencing, microarray technology, real-time polymerase chain reaction (PCR), and reverse transcription-quantitative real-time PCR.

2.5. Selection of sources of evidence

In some articles, the relevant criteria were met in the abstract section, but the content of the article was not found appropriate. Duplicate publications were done using software Mendeley Desktop version 1.19.8. The mechanism of sperm-induced infertility was examined in this work, although difficulties originating in other cells or tissues (Sertoli cells and Leyding cells origin) were not addressed. Additionally, several articles were dropped since the main content was in a foreign language but the abstract was in English.

2.6. Data charting process and data item

All data from articles included in the scoping review were extracted. In the extracted data, the name of the author, the year of the study, the hypothesis of the study, the molecular technique used and the study results were discussed. These extracted data were listed in the table using Microsoft Excel 2010.

2.7. Summarizing evidence

The extracted data were summarized and classified based on the name of the author, the year of the study, the hypothesis of the study, the molecular technique used for sncRNA and the study result.

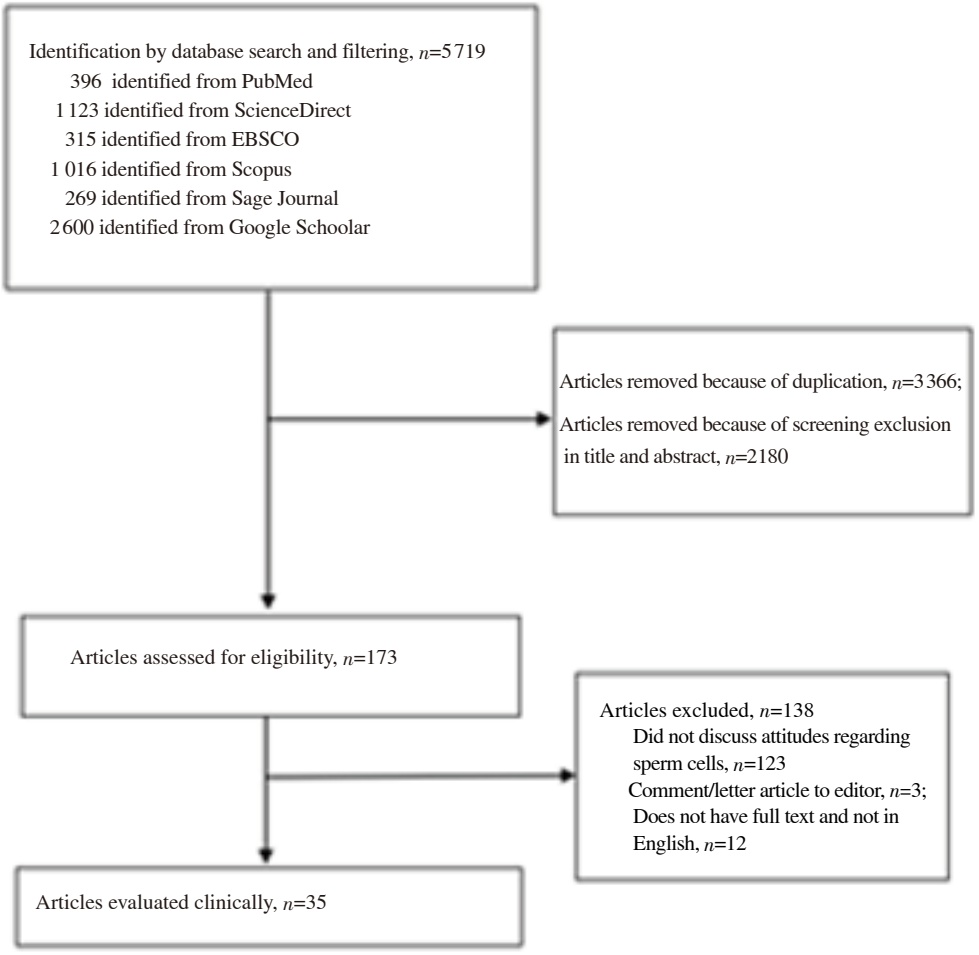

Figure 1. Flowchart of study screening process.

3. Results

3.1. Clinical and research consequences

Thirty-five articles in total were included in the scoping review shown in Figure 1. Infertility is linked to abnormal sncRNA expression in reproductive cells, according to a comprehensive assessment of descriptive and observational research (Table 1). As a result of the 35 literature in research, the sncRNAs that are causative factors in infertility were grouped under separate headings and evaluated.

3.2. miRNAs in Sertoli cells

Sertoli cells support germ cells, and different miRNAs control the production of molecules that control germ cell survival and development into spermatozoa. miR-30 became stated to be incredible, and miR-30 family expression in human testis tissue performed a role in reproductive development. In the studies carried out, three miRNAs (hsa-miR-34a, hsa-miR-34b, hsa-miR-34c) have low expression, and hsa-miR-34b, c promoters have hypermethylation in infertile men's sperm[16]. Another study found that miRNAs, miR-890, miR-892a, miR-892b, miR-891a, and miR-891b directly or indirectly affect sperm maturation and fertility[17].

3.3. miRNAs in Leydig cells

Leydig cells are essential and have been shown to be controlled by several miRNAs for androgen and sperm production. According to certain research, the ratio of Leydig cells in the developing testis is regulated by miR-140-5p/miR-140-3p, miR-155, miR-146a, miR-196a-2 expression in subfertile men and they are linked with subfertility[18,19].

3.4. miRNAs in azospermia

In some studies of azoospermic men, they found the highest fold reductions in miR-126, miR-10b, miR-191, miR-34c-5p, and miR-202-5p in comparison to controls. Besides, miR-202-5p was observed in Sertoli cells of fertile men but not in infertile men by immohistochemistry staining[20]. Zhao *et al* analyzed seminal plasma with high sperm DNA fragmentation from infertile men and found significant downregulation of miR-424[21]. Men have infertility due to irreversible DNA breaks brought on by the decline

Table 1. Data extraction from individual studies.

No.	Author	Year	Hypothesis	miRNA measurement technique	Results
1	Tsatsanis *et al*[18]	2015	Serum levels of micro-RNAs miR-155 and miR-146a associated with male fertility	RT-PCR (Real Time PCR)	miR-155 contribute to male fertility as a novel biomarker
2	Dabaja *et al*[20]	2015	Compare expression patterns of miRNAs in fertile and Sertoli cell only (SCO) men	RT-PCR	MicroRNA-202-5p has a different profile
3	Zhao *et al*[21]	2015	Found that during spermatogenesis, testicular miRNA is involved in sperm DNA damage.	RT-PCR	Sperm DNA damage is involved in miR-424/322
4	Meng *et al*[64]	2015	Metadherin in male fertility	RT-PCR	miR-16 and miR-19b reduced in the semen of infertile men
5	Zhou *et al*[57]	2020	The possible role of miRNAs associated with mitochondria in asthenozoospermia	RT-PCR	miR-101-3p, let-7b-5p) were significantly decreased, while sp-miR-151a-5p was increased in asthenozoospermia
6	Hong *et al*[52]	2016	SNPs is realeted with idiopathic male infertility: hsa-mir-146a rs2910164, hsa-mir-196a-2 rs11614913 and hsa-mir-499 rs3746444	RT-PCR	hsa-miR-196a-2 related with polymorphism and idiopathic male infertility.
7	Tang *et al*[29]	2016	The essential pathways involved in the spermatogenesis of miR-210 in patients with non-obstructive azoospermia (NOA)	RT-PCR	miR-210 was involved in spermatogenesis
8	Song *et al*[44]	2017	Analyzed miR-188- 3p expression in obstructive azoospermia and non-obstructive azoospermia patients	RT-PCR, Western blotting and immunohistochemical	miR-188-3p expression was lower in patients than control
9	Gou *et al*[51]	2017	Hiwi ubiquitination-deficient D-box mutations are detected in azoospermia patients	Immunostaining, Histological, Electron Microscopy,	Piwi as a causative factor in human infertility
10	Wang *et al*[50]	2018	Analysis of miRNA and piRNA profiles	RNA-Seq	piRNAs (hsa-piR-20830, hsa-piR-4731, hsapiR-6254, hsa-piR-419, hsa-piR-7152, hsa-piR-7548, hsa-piR-14195, hsa-piR-5026, hsa-piR-11482, hsa-piR-17765, hsa-piR-11873) are useful biomarkers for predicting NOA
11	Tang *et al*[40]	2018	Analyzedthe miRNA profile in the testis of post-cryptorchidopexy patients.	NGS RT-PCR	In cryptorchidism, the essential function of miRNAs
12	Dickson *et al*[28]	2018	Stress in early life changes the levels of different miRNAs in sperm	Microarray	34/449 miRNA influence spermatogenesis
13	Gholami *et al*[14]	2020	Determined the expression in infertile men of CRISP2 andmir-582	Bioinformatics studies, RT-PCR	Important increase in miR-582-5p expression in patients with teratozoospermia
14	Zhou *et al*[23]	2019	The research of the high-expression molecular mechanism of the *SEMG1* gene and its possible role in asthenozoospermia	Bioinformatics analysis	microRNA-525-3p (miR-525-3p) was found lower expressed
15	Xu *et al*[54]	2019	hsa-miR-34 family expression and methylation in infertile men's sperm samples	RT-PCR, MS PCR	Lower expression of hsa-miR-34a,b,c
16	Gao *et al*[43]	2019	Complete male sterility caused by over-expressed miR-10a in germ cells	RT-PCR	miR-10a-dependent meiotic process genetic regulation is important for both mouse and human development of male germ cells and spermatogenesis
17	Hua *et al*[15]	2019	miRNA may be useful for predicting and diagnosing in azoospermia	Bioinformatics analysis, RT-PCR	miR- 34b-5p and miR-10b-3p are used to be as a predictive biomarker of azoospermia
18	Vazguez *et al*[41]	2019	Investigated miRNA profiles in fertile and infertile man	RT-PCR	hsa-miR-1208/miR-942-5p, hsa-miR-34b-3p/ hsa-miR-93-3p are marker
19	Fang *et al*[42]	2019	Analysed miRNA changing expression profile sperm	RNA sequencing (RNA-Seq)	hsa-miR-10-5p, hsa-miR-182-5p, hsa-miR-22-3p, hsa-miR-378c, hsa-miR-449a, hsa-miR-486-5p, hsa-miR-507, hsa-miR-520a-3p, hsa-miR-520d-3p were downregulated, hsa-miR-199b-5p, hsa-miR- 3141, hsa-miR-374a-hsa-miR-6723-5p upregulated
20	Heidary *et al*[31]	2019	miRNA expression profile in spermatozoa in asthenozoospermic men	RT-PCR	Low expression of miR-4485-3p was related to idiopathic asthenozoospermia

Table 1. Data extraction from individual studies (continued).

No.	Author	Year	Hypothesis	miRNA measurement technique	Results
21	Belleannée [17]	2012	MicroRNAs (miRNAs) that play critical roles in regulation of gene expression	Microarray	miRNAs, miR-890, miR-892a, miR-892b, miR-891a, miR-891b directly or indirectly affect sperm maturation and fertility
22	Zhu *et al*[32]	2019	Investigated siRNA-mediated *CEP55* gene silencing on the proliferation spermatogonia ın azoospermia	Western blotting and qPCR	*CEP55* may play a key role in spermatogenesis and target for non-obstructive azoospermia
23	Dorostghoal *et al*[65]	2020	Characterization of the microRNA (miRNA) expression profile of normospermic patients in seminal plasma	NGS	897 human miRNAs were detected, miR-374b and miR-26b with significantly decreased expression
24	Joshi *et al*[30]	2022	lncRNAs in germ cells	Microarray	15 lncRNAs were different expression levels
25	Babakhanzadeh *et al*[45]	2020	Investigated *piRNAs* and *PIWI* genes in spermatogenesis to related Tudor domain-containing proteins.	RT-PCR, Western blotting	*TDRD* gene was downregulated and caused sperm infertility
26	Liu *et al*[33]	2020	Examines the roles of miRNAs in spermatogenesis	Microarray	miR-10b-3p and miR-34b-5p a predictive biomarker of azoospermia
27	Li *et al*[38]	2020	Role of miRNA in DNA fragmentation	RT-PCR	miR-26b and miR-374b could be used as the first biomarkers of increased sperm DNA fragmentation
28	Momeni *et al*[16]	2020	Evaluated the expression and methylation of hsa-miR-34 family in sperm samples of infertile men	RT-PCR	Lower expression of hsa-miR-34a, b, c and hypermethylates of hsa-miR-34b, c promoters in sperm samples of infertile men
29	Zhang *et al*[35]	2021	miR-423-5p related sperm motility	RT-PCR	High-level miR-423-5p inhibited sperm motility
30	Dorostghoal *et al*[47]	2022	miR-34c-5p related to sperm quality	RT-PCR	The miR-34c-5p transcript may be a good indicator of spermatozoa quality
31	Joshi *et al*[30]	2022	Use sperm transcriptome analysis to discover RNA-based indicators of male infertility.	Microarray	Compared to controls, 8100 genes were downregulated and 3588 genes were upregulated in cases
32	Oluwayiose *et al*[34]	2023	Vesicle-free seminal plasma may be related to poor sperm	Bioinformatics (FastQC)	Men with poor semen quality had altered expression levels of 57 seminal plasma extracellular vesicle non-coding RNAs
33	Conflitti *et al*[25]	2023	Describe the role of miR-34c-5p, and miR-449b-5p in sperm quality	Digital PCR (ddPCR)	miR-34c-5p and miR-449b-5p as a biomarkers for sperm quality
34	Wainstein *et al*[26]	2023	miRNAs may be used as biomarkers.	RNA deep sequencing	miRNA-370-3p was noticeably higher in azoospermic man
35	Abu-Halima *et al*[36]	2023	miRNAs (miR-15b-5p, miR-195-5p, miR-424-5p, and miR-497-5p) are related to sperm-associated antigen 7 (SPAG 7)	RT-qPCR	miR-424-5p, miR-497-5p, miR-195-5p, miR-424-5p, miR-497-5p, and miR-6838-5p showed significantly higher expression levels in oligoasthenozoospermic

NGG: next generation sequencing; RT-PCR: real time PCR; MS PCR: methylation-specific PCR.

in miR-16 and miR-19b expression levels. miR-18, miR-50, and miR38 are expressed by spermatozoa. When compared to fertile and azoospermia males, these miRNAs will adversely influence spermatogenesis[22]. The *SEMG1* gene is the main protein in semen. It participates in the formation of a gel matrix internally, which envelops important glandular secretions and ejaculated sperm. Lower expression of miR-525-3p targets the 3′-untranslated region of the *SEMG1* gene, resulting in higher expression of the *SEMG1* gene in azoospermia patients[23]. miR-34b-5p, miR-10b-3p, miRNA-370-3p, miR-34c-5p and miR-449b-5p can be used as predictive biomarkers of azoospermia[24–26].

3.5. miRNAs in asthenozoospermia

A study has been conducted in patients with asthenozoospermia, considering the idea that miRNAs associated with mitochondria can play an important role in controlling mitochondrial function. They showed let-7b-5p and hsa-miR-101-3p have low expression, but hsa-miR-151 has high expression of seminal miRNAs in asthenozoospermia patients compared with healthy controls[27].

Stress in childhood has long-term adverse health effects. Decreases in numerous miRNAs, including miR-34b/c in spermatozoa, have been linked to decreased fertility in humans. They reported the effect

of lifestyle stress on sperm cells, and they found reduced expression of miR-34 and 449[28,29]. While miR-582-5p expression decreased in asthenozoospermia groups, its expression level increased in teratozoospermia[30]. Heidary *et al* investigated the profile of miRNAs (miR-4485-3p/4484/4461 and 4463) in asthenozoospermic men's sperm. miR-4461 and miR-4484 were not detected in the control group. However, in asthenozoospermia, miR-4485-3p was greatly downregulated[31]. Zhu *et al* investigated both azoospermia patients to find that siRNA-mediated *CEP55* gene silencing affects proliferation and CEP55 may be a target for non-obstructive azoospermia (NOA)[32]. hsa-miR-27b-3p and hsa-miR-151a-5p were overexpressed in asthenozoospermic patients' semen samples, whereas hsa-miR-206 was down-expressed, according to a recent study[33]. Men with poor semen quality altered the expression levels of 57 seminal plasma extracellular vesicles non-coding RNAs[34]. Other studies demonstrated increased miR-423-5p expression in asthenozoospermia patients[35]. A study conducted in 2023 showed that miR-424-5p, miR-497-5p, miR-195-5p, miR-424-5p, miR-497-5p, and miR-6838-5p showed significantly higher expression levels in oligoasthenozoospermic men[36].

3.6. miRNAs in oligozoospermi

The discovery of more than 200 miRNAs in human sperm thus far suggests the importance of these RNAs in sperm maturation and morphogenesis[37]. Li *et al* found that miR-26b and miR-374b have significantly decreased expression and they can be used as the first biomarkers of increased sperm DNA fragmentation[38]. Muñoz *et al* investigated the expression profiling of miR-122, miR-34c-5p (B), and miR-449a (A) from fertile and oligozoospermic semen samples. They found high expression in let-7g/7b/7c, miR-21, miR-22, miR-148a, miR-30a, miR-320a, miR-2221, miR-375, miR-423-5p, miR-423-3p (except miR-30a) and low expression in miR-34b, miR-25, miR-122, miR-192, miR-152, and miR-335[39].

Tang *et al* indicated that post-cryptorchidopexy patients' miRNA expression levels are similar to those of NOA and obstructive azoospermic (OA) patients. They showed 297 downregulated and 152 upregulated miRNAs in OA compared with NOA patients[40]. hsa-miR-1208/miR-942-5p and hsa-miR-34b-3p/hsa-miR-93-3p have the potential to develop into novel biomarkers in sperm samples, thus helping to cure male infertility[41]. In an investigation, it was shown that spermatozoa had lower levels of a number of miRNAs, including the miR-34 family, which has been causally linked to lower human fertility. Fang *et al* analyzed 13 miRNAs were upregulated and 167 miRNAs were downregulated between groups[42]. Gao *et al* found that genetically conditioned overexpression of miR-10a causes full male infertility by stopping germ cells in the meiotic process. Their results indicated that the development and spermatogenesis of male germ cells in mice and humans are dependent on genetic control of the meiotic process[43]. Song *et al* analyzed miR-188-3p expression and found it was lower than in the control patients with comparative OA and NOA[44]. Babakhanzadeh *et al* sought to link piRNAs and *PIWI* genes in spermatogenesis to Tudor domain-containing proteins, and the *TDRD* gene was found to be downregulated, resulting in sperm infertility[45]. Abu-Halima *et al* investigated miR-23a/b-3p in oligoasthenozoospermia. They found that miR-23a/b-3p affected sperm morphology, count, and motility and had a negative correlation with ODF2 and UBQLN3[46]. miR-34c-5p is related to sperm quality and may help determine if a pregnancy will be successful[47]. In the study, 38 of 94 miRNAs were found to be related to spermatogenesis, and these miRNAs may have great potential to be used as indicators of infertility or sperm quality[48]. siRNA-mediated molecule research continues in infertility.

3.7. piRNAs in NOA

piRNAs play roles in the spermatogenetic process, and there is little information about the testicular tissues of infertile men. piRNA biogenesis has not been elucidated yet, and its mechanism of action on infertility is unknown. Studies on the subject are still ongoing[49]. Wang *et al* showed that 18324 piRNAs from homo sapiens were identified using small RNA-Seq technology from NOA patients, and 951 testicular piRNAs were significantly downregulated compared to the control group. They showed that 20 significantly differential piRNAs are useful biomarkers for predicting NOA[50]. Gou *et al* discovered that human Piwi (Hiwi) germline mutations cause histone-to-protamine exchange during human spermiogenesis to be impaired, which can cause azoospermia in humans. This research is the first to demonstrate how PIWI proteins affect human fertility[51]. Hong *et al* identified some piRNAs such as piR-31925, piR-31068, piR-43771, piR-30198 and piR-43773 as indicators for male infertility at the molecular level[52]. Nagirnaja *et al* showed that levels of the piRNA-processing proteins PIWIL1, PIWIL4, MYBL1, and TDRKH were significantly reduced. Also, men with PNLDC1 mutations also had significantly different piRNA length distributions and pachytene piRNA numbers[53,54].

3.8. Long noncoding RNA´s infertility

The mechanism and relationship of long noncoding RNAs to male infertility is still unknown. In recent studies, it has been mentioned that some lncRNAs may play basic roles during the formation of germ cells. Rolland *et al* identified 113 lncRNAs and 20 new genes that are transcribed during spermatogenesis[55].

Joshi *et al* identified lncRNAs regulating spermatogenesis in prenatal, postnatal, adult testicles and different germ cells. They found 15 lncRNAs (LINC00635, LINC00521, LINC00174, LINC00654, LINC00710, LINC00226, LINC00326, LINC00494, LINC00535, LINC00616, LINC00662, LINC00668, LINC00467, LINC00608, and LINC00658). FAM98B, CENPB, GOLGA6 family, *TPM2, RPGR, GNB5, CDKN2B, KCNQ10T1, LIN28A, CDKN2A, CDKN1A,*

CDKN1B, CDKN1C, EZH2, SUZ12, TAZ and *VEGFA* genes were a few of these lncRNAs' targets. In one study on male infertility in humans, three lncRNAs in particular (lnc32058, lnc09522, and lnc98497) showed specific and high expression in immotile sperm compared to normal motile sperm. LncRNA033862Drm, Tug1, Tsx, Spga-lncRNAs, NLC1-C, HongrES2, LncRNA-tcam1, Tesra, AK015322, Gm2044, and Mrhl are a few lncRNAs whose functions in spermatogenesis have been functionally confirmed[56]. Based on the GSE6872 dataset, Zhou *et al* discovered 101 lncRNAs and 1 754 mRNAs that showed differential expression across normospermic and teratozoospermic groups, including 33 downregulated and 68 upregulated genes[57].

As versatile regulators of gene expression and epigenetic events during spermatogenesis, small RNAs play a role. They can provide an important tool for evaluating appropriate methods for infertility diagnosis and treatment.

3.9. Biomarker in infertility

miRNAs have been shown to have the potential to be used as biomarkers throughout the world. In the area of *in vitro* fertilization, there are still a number of concerns to be addressed about the causes of male infertility, and new detection techniques must be created in order for better therapies to emerge. According to several studies, male infertility was associated with upregulated levels of miR-574-5p, miR-185, miR-122, miR-297, miR-1275, miR-373, and miR-193b, but miR-26a, miR-100, miR-19b, miR-512-3p, miR-16 and miR-23b levels were found to be normal[58]. Additional research has been done that has uncovered several microRNAs that might be used as biomarkers to identify male infertility. Although these compounds may not be able to completely replace proteins as biomarkers, their high efficiency and ability to detect diseases early make them ideal candidates for use as diagnostic aids.

miRNAs affect many genes and their clinical use is very difficult for patients. The fact that a single miRNA may affect several genes or that various miRNAs may be linked to the same gene makes it more challenging for researchers to do their study. The use of miRNA panels rather than individual miRNA molecules was one of the solutions presented for this problem. In order for these compounds to become routinely used in clinical practice, further joint effort is required[59].

Depending on the cause and the features of the affected person, treatments for infertility range from pharmacologic drugs to assisted reproductive technology. The major examination options for male fertility are sperm analysis, hormone examination, genetic testing, and testicular biopsy. Treatment options for male infertility include dietary adjustments, medication, surgery, and sperm regeneration. Male infertility is related to understanding all mechanisms of sperm maturation and fertilization. Infertility treatment, a fashionable illness, depends on the age, cause, and other characteristics of the individuals. Medication intends to fend against stress that is brought on by the body, mind, economy, and time.

Subsets of miRNAs might also have medical relevance as biomarkers. These biomarkers can be used to detect the presence of a pathology as well as the stage, progression, or genetic link that leads to it.

Idiopathic male infertility, which can reduce *in vitro* fertilization success, is thought to be caused by abnormal nuclear DNA packing and altered mRNA expression[60]. In beneficial situations, one miRNA biomarker could also be sufficient to determine a fitness result. However, in a number of situations, a separate panel of miRNAs are required for improved diagnostic sensitivity and/or specificity. These studies were carried out on preclinical animal models as well as human populations[61].

Recent years have seen significant advancements in the *in vitro* differentiation of male germ cells from pluripotent stem cells. Spermatogonial stem cells (SSCs) are responsible for continuing to produce male sperm in humans[62]. The basement membrane that protects the spermatogenesis' seminiferous tubules contains spermatogonial stem cells. Human SSC transplantation may be a successful treatment for male infertility. SSCs are actual stem cells that may pass on parental genetic information to their progeny. This information can also be used to address technological problems relating to human infertility[63].

4. Discussion

The creation of fully functioning spermatozoa and their successful arrival at the site of fertilization are both reliant on a number of physical, endocrine, and genetic variables that affect male fertility. To completely assess male reproductive potential and the causes of male infertility, fresh, specialized diagnostic methods are required[31]. MiRNA profiles of sperm from fertile and infertile males with or without morpho-functional sperm changes have been thoroughly studied in humans[44]. Sperm cells are extremely abundant in sncRNAs. These short RNAs could be crucial for post-fertilization development or play a significant part in the process of spermatogenesis[37]. These miRNAs could be useful indicators in assisted reproductive technique (ART) clinics for determining sperm quality. In situations of infertility utilizing ART and sperm molecular abnormalities with or without changes in the sperm profile, these miRNAs may potentially be employed as RNA therapies[19]. In situations of sperm molecular anomalies with or without changes to the semen profile, miRNAs can also be employed for RNA therapies of infertility utilizing ART[19–30].

An extremely distinct and specialized epigenetic landscape may be seen in the sperm epigenome. To evaluate the approximately 1 in 6 couples that encounter infertility, insightful questions concerning these epigenetic fingerprints and their capacity for prediction

must be raised[41]. About half of the cases of infertility in couples who do encounter it have a male element. The current study has many strengths along with a few limitations. Unfortunately, there is a severe shortage of efficient diagnostic tools in the field of male infertility, leaving doctors with no information on which to base data-driven treatment regimens. A significant hurdle that has to be overcome is developing a better knowledge of how these sperm intrinsic elements regulate reproductive and developmental processes[59]. In light of all of this data and the startlingly high frequency of male infertility[47], it is clear that more effective diagnostic methods are required for male infertility. There are many causes of male infertility that have not yet been discovered. In addition, there are many infertile couples who are not clinically evaluated and the causes of infertility are not revealed due to insufficient research. Numerous studies have discovered sperm epigenetic changes that seem to be clinically significant, which may be helpful in the diagnosis of infertility and the enhancement of pregnancy outcomes. Intriguingly, the projected gene targets of the differentially expressed sncRNAs are engaged in biological processes that have an impact on sperm morphology, sperm motility, and/or spermatogenesis. Numerous targets of encoded proteins have also been linked to sperm that are defective or have poor motility. Understanding the molecular functions sncRNAs which play in reproductive processes will be essential for understanding miRNA expression and function[56,64,65]. Numerous experts think that further study of these numerous epigenetic pathways might yield insightful predictions with great force.

5. Conclusions

In this review article, sncRNAs that are effective on infertility are evaluated. Consequently, given the increasing cases of idiopathic infertility, germ cell development, low sperm quality and living conditions that make up the etiology of male infertility, idiopathic male infertility remains one of modern medicine's most difficult problems to solve. It contributes greatly to germ cell development and differentiation of siRNAs, miRNAs and piRNAs. The irregularity of specific sncRNAs results in infertile men and their regulating mechanisms have not been clarified. Understanding the factors behind male infertility and sterility can help understand their genesis. sncRNAs can act as biomarkers for the diagnosis and treatment of these reproductive diseases. We can also select high-quality sperm for assisted reproductive therapy by sorting specific sncRNAs. To shed fresh insight on the crucial functions of sncRNA in spermatogenesis and fertility, further investigations and research will be carried out in this area.

Conflict of interest statement

The authors declare no conflicts of interest.

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Authors' contributions

Eroglu Onur provided the definition, concept, and design of intellectual material. Investigation, literature search, and data analysis were all done by Kaya Cakır Hacer. Kaya Cakır Hacer and Eroglu Onur wrote, edited, and reviewed the paper.

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