MicroRNAs: Recent insights towards their role in male infertility and reproductive cancers

Muhammad Babar Khawar[1,2,3*], Rabia Mehmood[1], Nabila Roohi[1*]

[1]Molecular Physiology/Endocrinology Laboratory, Department of Zoology, University of the Punjab, Lahore, Pakistan, [2]State Key Laboratory of Stem Cells and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China, [3]University of Chinese Academy of Sciences, Beijing, China

ABSTRACT

Spermatogenesis is a tightly controlled, multi-step process in which mature spermatozoa are produced. Disruption of regulatory mechanisms in spermatogenesis can lead to male infertility, various diseases of male reproductive system, or even cancer. The spermatogenic impairment in infertile men can be associated with different etiologies, and the exact molecular mechanisms are yet to be determined. MicroRNAs (miRNAs) are a type of non-protein coding RNAs, about 22 nucleotides long, with an essential role in post-transcriptional regulation. miRNAs have been recognized as important regulators of various biological processes, including spermatogenesis. The aim of this review is to summarize the recent literature on the role of miRNAs in spermatogenesis, male infertility and reproductive cancers, and to evaluate their potential in diagnosis, prognosis and therapy of disease. Experimental evidence shows that aberrant expression of miRNAs affects spermatogenesis at multiple stages and in different cell types, most often resulting in infertility. In more severe cases, dysregulation of miRNAs leads to cancer. miRNAs have enormous potential to be used as diagnostic and prognostic markers as well as therapeutic targets in male infertility and reproductive system diseases. However, to exploit this potential fully, we need a better understanding of miRNA-mediated regulation of spermatogenesis, including the characterization of yet unidentified miRNAs and related regulatory mechanisms.

KEY WORDS: Infertility; microRNAs; miRNAs; noncoding RNAs; reproductive cancers; spermatogenesis

DOI: http://dx.doi.org/10.17305/bjbms.2018.3477 *Bosn J Basic Med Sci. 2019;19(1):31-42. © 2018 ABMSFBIH*

INTRODUCTION

About 10–15% of couples trying to conceive face infertility and male factors contribute to approximately 50% of these cases [1]. In the past several decades, considerable efforts have been made to elucidate the etiology of male infertility, however, the causative factors are still not completely understood. For example, in men with non-obstructive azoospermia (NOA) undergoing testicular sperm extraction at one center, the rates of sperm retrieval, fertilization and clinical pregnancy were lower and the frequency of chromosomal abnormalities/Y chromosome microdeletion was higher in those that had uniform maturation arrest (MA) and normal follicle-stimulating hormone (FSH), compared to other men with NOA [2]. Thus, clarifying the pathogenesis of MA may contribute to better outcomes in these patients [1].

*Corresponding authors: Muhammad Babar Khawar and Nabila Roohi, Molecular Physiology/Endocrinology Laboratory, Department of Zoology, University of The Punjab New Campus, Canal Bank Road, Lahore, Pakistan. Phone: +92-3214575910, +92-3334053953. E-mail: babarkhawar@yahoo.com, nabilaruhi@gmail.com

Submitted: 09 April 2018/Accepted: 20 May 2018

Spermatogenesis is a multi-step process in which undifferentiated (diploid) spermatogonial stem cells (SSCs) develop into mature and motile (haploid) sperm cells called spermatozoa. The distinct stages of spermatogenesis include [3,4]: 1) The mitotic division of the SSCs which results in two types of spermatogonia; type A cells are reserved for the renewal of SSCs, while type B cells 2) further differentiate into (diploid) primary spermatocytes. The primary spermatocytes undergo meiosis I resulting in two (haploid) secondary spermatocytes and these cells, through meiosis II, produce four (haploid) spermatids. 3) In the final stage called spermiogenesis, spermatids undergo numerous morphological changes, such as the formation of the acrosome, nuclear condensation, development of the flagellum and reorganization of the cytoplasm, to give rise to mature spermatozoa (Figure 1).

Early studies indicated the role of increased apoptosis rate of spermatogonia in MA and hypospermatogenesis in infertile men, based on histological evidence and detection of apoptotic DNA fragmentation [5]. On the basis of Ki-67 and proliferating cell nuclear antigen (PCNA) immunoreactivity in testicular biopsies of oligozoospermic men with mixed atrophy, Steger at al. [6] showed that low spermatogenic efficiency in infertile men is not only associated with meiotic and postmeiotic events but also

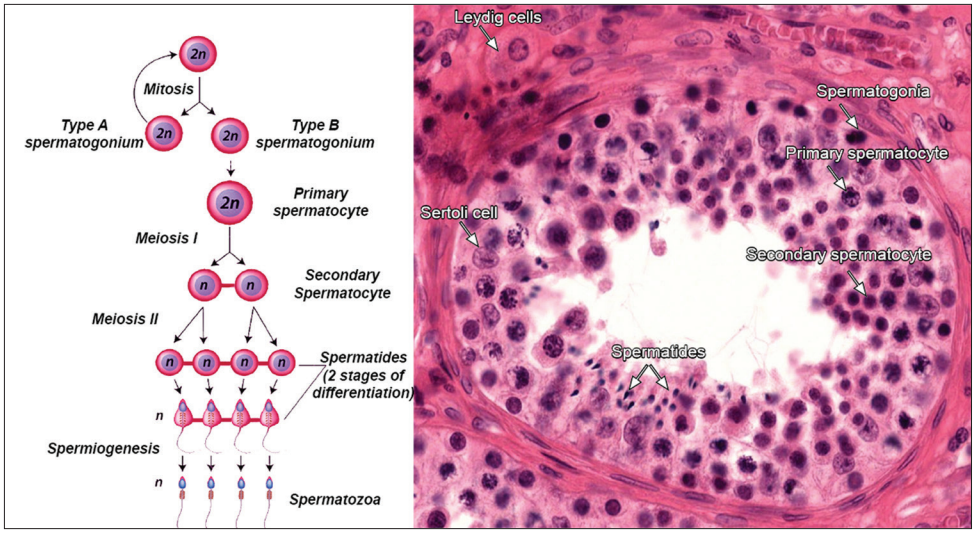

FIGURE 1. A schematic representation of different stages of spermatogenesis in which mature and motile spermatozoa are produced. Spermatogonial stem cells (SSCs) develop from gonocytes in the postnatal testis, which arise from primordial germ cells (PGCs) during fetal development. In adult males, the mitotic division of the SSCs results in two types of spermatogonia; type A cells are reserved for the renewal of SSCs, while type B cells further differentiate into (diploid) primary spermatocytes. The primary spermatocytes undergo meiosis I resulting in two (haploid) secondary spermatocytes and these cells, through meiosis II, produce four (haploid) spermatids. In the final stage called spermiogenesis, spermatids undergo numerous morphological changes, such as the formation of the acrosome, nuclear condensation, development of the flagellum and reorganization of the cytoplasm, to give rise to mature spermatozoa.

with a functional impairment of spermatogonia [6]. In addition, Maymon et al. [7] suggested that in azoospermic cases characterized by homogenous appearance of spermatocyte MA, the spermatogenic failure was a consequence of meiosis impairment (low rate of bivalent formation) rather than a malfunction of spermatogonial activity [7]. Similarly, other studies demonstrated an increased occurrence of recombination defects and meiotic abnormalities in men with NOA [8,9]. Overall, the above-described studies indicate that the spermatogenic impairment in infertile men can be associated with various etiologies, and the exact molecular mechanism underlying the regulation of spermatogonial proliferation is yet to be determined.

The aim of this review is to summarize the recent findings regarding the role of microRNAs in spermatogenesis, male infertility and reproductive cancers, and to evaluate their potential in diagnosis, prognosis and therapy of disease.

MICRORNAS: A HISTORICAL PERSPECTIVE

It is now well-established that only a small fraction of the human genome (only 1–2%) is comprised of protein-coding sequences, while the rest is made up of regulatory DNA sequences, noncoding RNA (ncRNA) sequences, introns, transposons, repeats, and of sequences whose function has yet to be determined. ncRNAs include transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small RNAs such as microRNAs (miRNAs) and small interfering RNA (siRNAs), and long ncRNAs. miRNAs are small ncRNAs of about 22 nucleotides in length, involved in the post-transcriptional regulation through translational repression, cleavage or destabilization of the target mRNA [10-14].

Since the discovery of the first small regulatory RNAs *lin-4* and *let-7* in *Caenorhabditis elegans*, which were later classified as miRNAs, more than 1500 different miRNA genes have been identified in the human genome [15-18]. It is predicted that over 60% of human protein-coding genes are conserved targets of miRNAs [19].

In the first stages of miRNA transcription, the precursor molecules of miRNAs (primary transcripts, pri-mRNAs) are transcribed in the form of long hairpin structures, either by RNA polymerase (pol) II from independent genes (intergenic, otherwise noncoding regions) or from introns or untranslated region (UTR) of protein-coding genes. The transcription of miRNA from independent genes is regulated in a similar way as in protein-coding genes; i.e., RNA pol II, promoters, enhancers, silencing elements and various transcription factors are required to generate pri-miRNAs with a 5′ 7-methylguanosine cap and 3′ polyadenylated [poly(A)] tail. In addition, nearly half of all miRNA genes are arranged in close proximity to each other (within 50 kb of another miRNA gene) and these clusters, ranging from 2 to 46 miRNAs (the largest, chromosome 19 miRNA cluster [C19MC] in primates), can be transcribed as polycistronic units [20]. After the initial transcription, pri-miRNAs undergo two processing steps catalyzed by two enzymes from ribonuclease III (RNase III) superfamily, Drosha and Dicer. First, the nuclear Drosha-DGCR8 complex processes the newly transcribed pri-miRNA into an ~70-nucleotide intermediate termed a precursor (pre)-miRNA. This pre-miRNA is then transported

to the cytoplasm where it is processed by the Dicer-TRBP complex resulting in an ~20-bp miRNA: miRNA* duplex (passenger strand), and one strand of this duplex is the mature miRNA. Following Dicer processing, the miRNA duplex is loaded onto an AGO protein to form the miRNA-induced silencing complex (miRISC). The mature miRNA strand is incorporated into the miRISC, while the other (star) strand is released from the duplex and degraded. Finally, as a part of (activated) miRISC, miRNA binds to the 3′-UTR of target mRNA by recognizing mRNA sequences that are complementary to miRNA seed region (residues 2–8 at the 5′ end of miRNA) and leads to either translational repression or mRNA deadenylation and degradation of the target gene (Figure 2) [21].

MicroRNAs: A KEY PLAYER IN THE REGULATION OF BIOLOGICAL PHENOMENA

miRNAs contribute to the regulation of various biological processes such as embryonic development, cell differentiation, cell cycle, cell growth and apoptosis [22-24], and dysregulation of miRNA functions can lead to the development of disease. For instance, psoriasis, cardiovascular disease, schizophrenia, diabetes, cancer, kidney disease and muscular disorders have been associated with impaired biosynthesis, mutations or improper regulation of miRNAs and their targets [25-31].

Different experimental and computational approaches for identifying miRNA targets, mostly based on the patterns of complementary base pairing between miRNA and target mRNA, have demonstrated that a single miRNA can regulate the expression of hundreds of target genes [32].

miRNAs are essential for early embryogenesis and cellular differentiation, where their activity is regulated by other proteins/complexes depending on the stage. For example, miRNAs are involved in the maintenance of primordial germ cells (PGCs) through the interaction with RNA-binding protein (RBP) Dead end (Dnd1), which counteracts the suppressive function of miRNAs by preventing its association with the target mRNA [33].

Previous research demonstrated that human spermatozoa retain various RNAs (including miRNAs) after the completion of spermatogenesis; moreover, changes in the expression of spermatozoal RNAs have been associated with male infertility. Different molecular techniques such as reverse transcription polymerase chain reaction (RT-PCR), real-time PCR, complementary DNA (cDNA) cloning, microarrays, DNA and RNA sequencing are employed for the detection and analysis of these RNAs in sperm samples [34].

miRNAs are present in Leydig cells, Sertoli cells and spermatogonia within the testis, as well as in mature spermatozoa, and have a vital role in testicular development, function and spermatogenesis. The presence of miRNAs in spermatozoa confirms their role in early embryonic development after fertilization, including epigenetic effects and chromatin restructuring [35].

ROLE OF MICRORNAS IN SPERMATOGENESIS AND MALE INFERTILITY

As post-transcriptional repressors, miRNAs are important in the regulation of spermatogenesis, and the dysregulation of miRNAs has detrimental effects on male fertility [4]. Generally, the enzymes involved in the biosynthesis of miRNA- and RNA interference (RNAi)-related pathways, such as Dicer protein, appears to be crucial for normal development, as the loss of Dicer during early embryonic development in mice leads to lethality [36]. To determine the exact roles of small ncRNAs in the male reproductive system, various mouse models have been developed using the *Cre/Lox* system, which enables conditional manipulation of a target gene (e.g., to knock out, insert, replace, activate or modify the expression of a gene) [37-45]. Cell-specific deletion of the Dicer gene (*Dicer1*) in mouse epididymal cells affected differentiation of the epididymal epithelium, lipid synthesis and sperm maturation [37,38]; in Sertoli cells had a major impact on testicular proteome [40], resulted in complete absence of spermatozoa and progressive testicular degeneration [45]; in male germ cells impaired haploid spermatid differentiation [39] and led to apoptosis and/or spermatogenic failure in the meiotic and haploid phases [41,42,44]; and in PGCs affected their proliferation and spermatogenesis at early stages [43]. In most of these cases the sum of the defects led to male infertility. As demonstrated by Björkgren et al. with a conditional knock-out of *Dicer1* in the proximal mouse epididymis (*Dicer1*$^{fl/fl}$; *Defb41*$^{iCre/wt}$ [Dicer1 cKO] mice) [37,38], not only that the sperm production depends on the miRNA biogenesis, but also the sperm maturation in the principal cells of the epididymis is impaired in the absence of mature miRNAs. The sperm cells from Dicer1 cKO mice had a reduced ability to bind to and fertilize the oocyte *in vitro* [37].

A large number of studies have been carried out to determine the roles of individual miRNAs expressed during spermatogenesis, and their effects on male fertility [46-48]. In mice, deletion of two functionally related, miR34b/c and miR-449, clusters encoding five miRNAs impaired both meiosis and spermatozoa maturation, resulting in oligoasthenoteratozoospermia (OAT) [47]. Another study showed that simultaneous inactivation of these two miRNA clusters in double-knockout (KO) mice leads to developmental defects and infertility in males and females, which was correlated with dysregulation of more than 200 genes targeted by

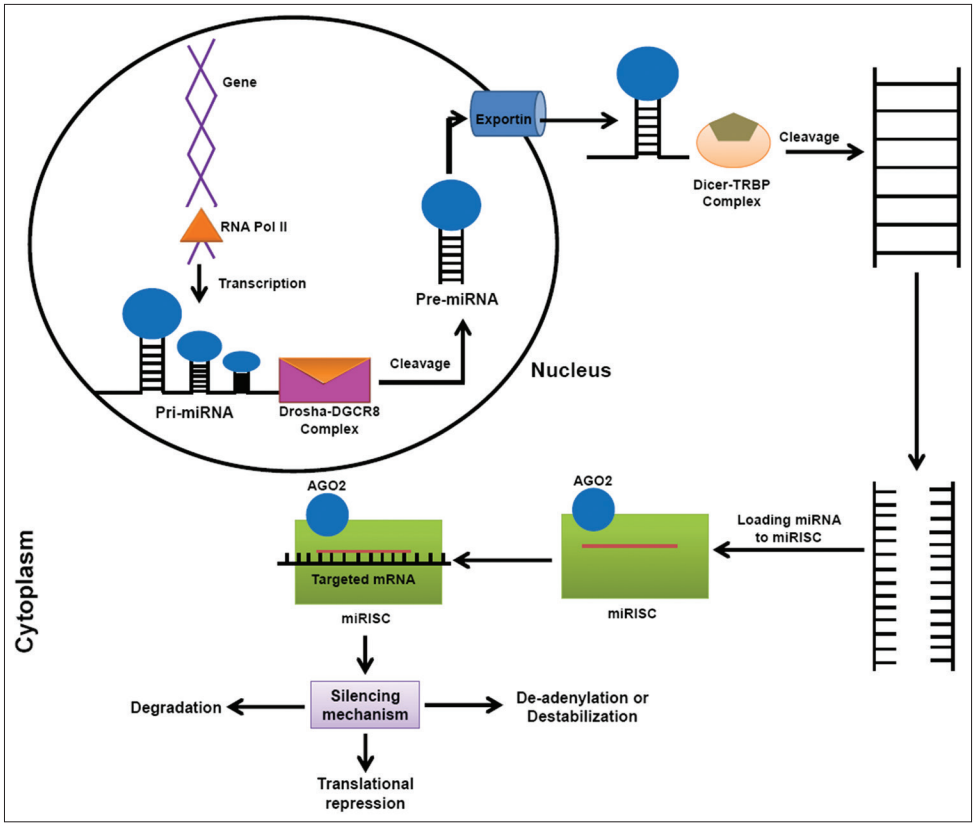

FIGURE 2. MicroRNA (miRNA) biogenesis and their role in post-transcriptional regulation. In the first stages of miRNA transcription, the precursor molecules of miRNAs (primary transcripts, pri-mRNAs) are transcribed in the form of long hairpin structures, either by RNA polymerase (pol) II from independent genes (intergenic, otherwise noncoding regions) or from introns or untranslated region (UTR) of protein-coding genes. The transcription of miRNA from independent genes is regulated in a similar way as in protein-coding genes; i.e., RNA pol II, promoters, enhancers, silencing elements and various transcription factors are required to generate pri-miRNAs with a 5′ 7-methylguanosine cap and 3′ polyadenylated [poly(A)] tail. After the initial transcription, pri-miRNAs undergo two processing steps catalyzed by two enzymes from ribonuclease III (RNase III) superfamily, Drosha and Dicer. First, the nuclear Drosha-DGCR8 complex processes the newly transcribed pri-miRNA into an ~70-nucleotide intermediate termed a precursor (pre)-miRNA. This pre-miRNA is then transported to the cytoplasm where it is processed by the Dicer-TRBP complex resulting in an ~20-bp miRNA: miRNA* duplex (passenger strand), and one strand of this duplex is the mature miRNA. Following Dicer processing, the miRNA duplex is loaded onto an AGO protein to form the miRNA-induced silencing complex (miRISC). The mature miRNA strand is incorporated into the miRISC, while the other (star) strand is released from the duplex and degraded. Finally, as a part of (activated) miRISC, miRNA binds to the 3′-UTR of target mRNA by recognizing mRNA sequences that are complementary to miRNA seed region (residues 2–8 at the 5′ end of miRNA) and leads to either translational repression or mRNA deadenylation and degradation of target gene [21].

miR34 and miR-449 miRNAs [48]. Comparably, double KO of miR-200b and miR-429 resulted in anovulation and reduced fertility in female mice, probably by affecting the hypothalamo-pituitary-ovarian axis. On the other hand, in double KO male mice abnormalities in the testis or fertility defects were not observed [46]. Targeted deletion of specific miRNAs is a key step in understanding their function *in vivo.* However, a deletion of a single gene usually does not produce a recognizable phenotypic effect because of the compensatory action of related miRNAs.

Analyzing miRNA expression profiles in spermatozoa of healthy fertile men, Salas-Huetos et al. [49] showed that 221 miRNAs were consistently detected in all 10 individuals; among the 10 most expressed miRNAs hsa-miR-34b-3p was associated with the regulation of male meiosis via the E2 factor-retinoblastoma protein (E2F-pRB) pathway, hsa-miR-132-3p with cell cycle progression through *MYC* activation, and hsa-miR-191-5p with sperm differentiation. Moreover, hsa-miR-30b-5p, hsa-miR-200c-3p, hsa-miR-30c-5p, hsa-miR-19b-3p, hsa-miR-891a and hsa-miR-375 were related to cancer development and/or aging [49]. The first report on altered miRNA expression in patients with NOA showed that 154 miRNAs were differentially downregulated and 19 upregulated in NOA patients compared to fertile men [50]. In another study, 197 miRNAs in Sertoli cells, 68 in mixed atrophy, and 46 in spermatocyte arrest group were differentially expressed compared to samples with normal spermatogenesis. Among these, miRNAs from miR-449 and miR-34 families (e.g., hsa-miR-449a, hsa-miR-34b*, hsa-miR-34b, and hsa-miR-34c-5p), which are preferentially expressed in the testis, were validated by RT-qPCR and implicated in apoptosis, cell proliferation and differentiation according to bioinformatics analysis [51]. Overall, these results confirm the role of miRNAs in the regulation of male germ and somatic cells, and any change in

their expression may lead to reproductive abnormalities [51]. Similarly, other studies reported up/downregulation of different miRNAs in asthenozoospermic, oligoasthenozoospermic, NOA, and/or teratozoospermic versus normozoospermic men, indicating their potential use in the diagnosis and treatment of male subfertility/infertility (Table 1) [52-60].

In addition, specific genes known to be involved in spermatogenesis and to affect male fertility were experimentally shown to be regulated by miRNAs. Zhou et al. [61] demonstrated that the protein expression of cysteine-rich secretory protein 2 (CRISP2) is downregulated by miR-27a in asthenoteratozoospermia (ATZ) patients. Namely, they observed a negative correlation between miR-27a (high) and CRISP2 protein (low) expression in ATZ patients and showed that miR-27a binds to the *CRISP2* 3′-UTR mediating its translational repression. CRISP2 protein is important for sperm flagellar motility, acrosome reaction and sperm-egg fusion [61]. In oligospermic infertile patients, estrogen receptor-alpha (ERα) levels were significantly decreased compared to fertile men, while the levels of miR-100 and let-7b miRNAs were significantly higher in oligospermic vs. control group, indicating their involvement in the regulation of estrogen signaling. The important role of estrogen and estrogen receptors in the function of the male reproductive system has been indicated by experimental studies on mice, as well as genetic and clinical studies on infertile/subfertile men [62-64]. In severe oligozoospermic patients, phosphatidylinositol-specific phospholipase C, X domain containing 3 (PLCXD3), a protein which is normally expressed in the late stages of spermatogenesis, was reported to be downregulated by miR-34c-3p [65]. Another study demonstrated that, in sterile men with NOA (MA or hypospermatogenesis), miR-210 was significantly upregulated compared to control OA group, and apparently affected spermatogenesis by targeting insulin-like growth factor 2 (IGF2). Generally, IGFs control biological processes by regulating cell survival, growth, proliferation and differentiation, and are important for proper development/function of the testis, including the regulation of Sertoli cell number and testis size [66]. A summary of studies on the role of miRNAs in spermatogenesis is presented in Table 2.

MICRORNAS AS A POTENTIAL BIOMARKER OF MALE INFERTILITY

In addition to cellular microenvironment, including Sertoli, Leydig cells, spermatogonia and mature spermatozoa, miRNAs can be detected in seminal and other body fluids, and these are called circulating or extracellular miRNAs. In normal physiological conditions, levels of extracellular miRNAs are stable in body fluids, and alterations in the expression of intra and extracellular miRNAs may indicate the presence of pathophysiological processes [35].

Seminal plasma is a complex fluid composed of secretions from the testis, epididymis, seminal vesicles, ampullae, prostate and bulbourethral glands. The presence of extracellular miRNAs in seminal plasma, either within extracellular vesicles (EVs) such as exosomes and microvesicles, or possibly associated with protein complexes, suggests their use as non-invasive biomarkers for spermatogenesis impairment and infertility. Both extra and intracellular miRNAs (from spermatozoa) can be isolated by non-invasive procedures and detected with routine RNA techniques such RT-qPCR and miRNA microarray, providing some advantages over conventional, invasive diagnostic procedures (e.g., prostate and testicular biopsies) [35,59,67].

Several studies reported differential expression of stable, extracellular miRNAs in seminal plasma from infertile men compared to fertile controls [57,59]. For instance, Wang et al. [59] showed that miR-34c-5p, miR-122, miR-146b-5p, miR-181a, miR-374b, miR-509–5p, and miR-513a-5p were markedly decreased in azoospermic but increased in asthenozoospermic men, compared to controls. Moreover, their ROC curve analyses indicated that the seminal plasma concentrations of these miRNAs could accurately distinguish infertile patients from fertile controls as well as azoospermic from asthenozoospermic men [59]. By analyzing miRNA profiles from normal, vasectomized and vasovasostomized donors, Belleannée et al. [68] investigated to what extent vasectomy affects miRNA expression in the epididymis and seminal microvesicles (SMVs), and whether miRNA changes in SMVs are reversible with vasovasostomy. They showed that vasectomy significantly changed the expression of epididymal miRNAs as well as affected the expression of 118 miRNAs in SMVs, including miRNAs contained in epididymosomes. Out of 118 altered miRNAs in SMVs, the expression changes were reversible for 52 and non-reversible for 66 miRNAs following vasovasostomy [68]. Most of the miRNAs responsive to vasectomy and its reversal [68] were also associated with bovine epididymosomes [69]. The exception are the members of miR-888 cluster family (i.e., miR-888, mir-890, miR-891a/b, and miR-892a/b), which are absent in all mammalian species except primates [69]. This miR-888 cluster is located on the X chromosome and expressed almost exclusively in epididymal tissues [69,70]. The rapid evolution of this cluster and tissue-specific expression suggest that it had an important role in the development of primate-specific epididymal functions. Computational functional analyses supported these assumptions as the predicted targets of miR-888 cluster family were related to epididymal morphogenesis, sperm maturation and immune cell functions; nevertheless, these findings need to be confirmed *in vivo* [69,71].

In mammals, sperm cells deliver to oocytes not just the paternal genome but also the paternal epigenome, which includes protein factors, DNA methylation patterns, retained

TABLE 1. Up and downregulation of various microRNAs (miRNAs) in subfertile and infertile men

Types/Number of patients	Tissue used for miRNA isolation	miRNA analysis method	Upregulated miRNAs	Downregulated miRNAs	Putative functions of miRNA in different cellular processes	Reference
Non-obstructive azoospermia (n=100), fertile control (n=100)	Seminal plasma	TaqMan low density array followed by TaqMan quantitative reverse transcription PCR (RT-qPCR) assay	miR-141, miR-429, and miR-7-1-3p	-	Biomarkers for spermatogenic failure	[52]
Normozoospermia (n=9), asthenozoospermia (n=9), and oligoasthenozoospermia (n=9)	Semen	Microarray with RT-qPCR validation	50 miRNAs upregulated	27 miRNAs downregulated	Play a role in spermatogenesis	[53]
Subfertile and non-obstructive azoospermia (n=80), fertile control (n=90)	Semen	RT-qPCR	hsa-miR-429	hsa-miR-34b*, hsa-miR-34b, hsa-miR-34c-5p, and hsa-miR-122	Biomarkers for the assessment of male infertility	[54]
Asthenozoospermia [A] (n=10), teratozoospermia [T] (n=10), and oligozoospermia [O] (n=10)	Semen	Taqman RT-qPCR	A group (26), T group (11), O group (3)	A group (6), T group (8), O group (15)	Spermatogenesis	[55]
Oligoasthenozoospermia, subfertile (n=12), normozoospermia (n=12)	Seminal plasma	Microarray with RT-qPCR and Western blot validation	7 miRNAs	29 miRNAs	Mechanisms underlying male infertility	[56]
Oligozoospermia (n=48), non-obstructive azoospermia (n=48), and fertile controls (n=48)	Seminal plasma	Quantitative RT-qPCR	miR-19b and let-7a	-	Aberrant overexpression may be an indicator of spermatogenic failure	[57]
Infertile men (n=289) and age-matched fertile control (n=168)	Seminal plasma	Solexa sequencing technology followed by stem-loop RT-qPCR	miR-34c-5p, miR-122, miR146b-5p, miR-181a, miR-374b, miR-509-5p, and miR-513a-5p (in asthenozoospermia)	miR-34c-5p, miR-122, miR146b-5p, miR-181a, miR-374b, miR-509-5p, and miR-513a-5p (in azoospermia)	Role in infertility	[59]
	Human cancer cell line	MiRNA transfection, RT-qPCR, Western blot analysis	miR-27a and miR451	-	Involved in the regulation of drug resistance mediated by multidrug resistance 1 (MDR1)/ P-glycoprotein	[60]
Asthenoteratozoospermia (n=20) and normozoospermia (n=20)	Semen	RT-qPCR and Western blot	miR-27a	-	High miR27a expression and low cysteine-rich secretory protein 2 (CRISP2) protein expression were associated with low progressive sperm motility, abnormal morphology, and infertility	[61]

histones and noncoding RNAs [72]. Yuan et al. [72] investigated the role of two miRNA clusters including five miRNAs, miR-34b/c and miR-449a/b/c, in the fertilization, first cleavage division and subsequent preimplantation in mice. These miRNAs are generally present in sperm but absent in oocytes, and all five were required for normal spermatogenesis and male fertility, i.e., miR-34b/c and miR-449 double miR-dKO mice displayed severe spermatogenic disruptions and OAT characteristics. However, it appears that these miRNAs are dispensable for fertilization and preimplantation development, as both miR-34b/c- and miR-449-null male mice were fertile; the intracytoplasmic injection of either miR-34b/c- or miR-449-null sperm and round spermatid injection of miR-dKO sperm led to normal fertilization and normal birth rate. Overall, these results indicate the functional redundancy of the two miRNA clusters in mice [72].

TABLE 2. Recent findings on the role of microRNAs (miRNAs) in spermatogenesis

Serial number	microRNA	Methodology	Findings	Reference
1	miR-17-92 cluster (miR-17, miR-18a, and miR-20a)	Real-time polymerase chain reaction (real-time PCR)	miR-17-92 cluster is upregulated during spermatogenesis in normal conditions. Targeted disruption in the testes of adult mice leads to severe testicular atrophy, empty seminiferous tubules, and decreased sperm production.	[95]
2	miR-17-92 (Mirc1) and miR-106b-25 (Mirc3) cluster	miRNA array analysis and quantitative reverse transcription PCR (RT-qPCR)	Male germ cell-specific miR-17-92 knockout mice shows small testes, a lower number of epididymal sperm, and mild defect in spermatogenesis. Deletion of miR-17-92 in male germ cells dramatically increases expression of miR-106b-25 cluster in the germ cells.	[96]
3	miR-290-295 cluster	RT-PCR	In surviving miR-290-295-deficient mouse embryos, infertility in females (but not in males) results from a defect in migrating primordial germ cells. The defect occurs equally in male and female mutant animals, but male mice are able to recover from the initial germ cell loss.	[97]
4	miR-146a	Real-time PCR	Inhibition of miR-146a promotes differentiation of mouse spermatogonia.	[98]
5	miR-221/222	RT-PCR	miR-221/222 plays a crucial role in maintaining the undifferentiated state of mammalian spermatogonia.	[99]
6	miR-21	RT-qPCR	Transient inhibition of miR-21 in spermatogonial stem cells (SSC)-enriched germ cell cultures increased the number of germ cells undergoing apoptosis in mice.	[100]
7	miR-135a	Microarray analysis and subsequent RT-qPCR	miR-135a was expressed at a lower level in undescended testes. miR-135a and forkhead box protein O1 (FOXO1) localized to spermatogonial stem cells in rats. miR-135a transfection into spermatogonia in vitro resulted in downregulation of FOXO1 expression.	[101]
8	miR-449	Microarray and real-time PCR	miR-449 clusters targets E2 factor-retinoblastoma protein (E2F-pRb) pathway and were preferentially expressed in the mouse testis; their levels were drastically upregulated upon meiotic initiation during testicular development, and in adult spermatogenesis.	[102]
9	miR-469	Microarray analysis and RT-qPCR	Plays a critical role in spermiogenesis and chromatin remodeling in mice by targeting transition protein 2 (TNP2) and protamine 2 (PRM2).	[103]
10	miR-34b/c and miR-449a/b/c	Real-time PCR	miR-34b/c and miR-449 double knockout (miR-dKO) male mice were infertile.	[104]

During mammalian spermatogenesis, a large number of mRNAs undergo post-transcriptional and translational regulation in rapidly dividing and differentiating germ cells [73], and it is now clear that miRNAs have an essential role in these regulatory mechanisms. Moreover, some miRNAs, such as hsa-miR-372 and hsa-miR-373 [74], have been identified as potential oncogenes and implicated in the development of human testicular germ cell tumors (TGCTs).

MICRORNAS IN REPRODUCTIVE CANCERS

As indicated in previous sections, miRNAs are predicted to regulate the expression of about 60% protein-coding genes in the human genome, hence their proper functioning is pivotal for normal cellular development [75]. In addition, dysregulation of miRNAs expression can lead to the development of several cancer types, including testicular cancer [75,76].

Previous studies identified specific roles of several miRNAs in spermatogenesis, e.g., miR-18a, miR-122a and miR-34 family, which were implicated in the regulation of germ cell function, cell fate determination, maintenance of undifferentiated stem cell populations, as well as cell differentiation during spermatogenesis [76,77-79]. Characterization of specific roles of miRNAs in germ cell development can be useful for the assessment of normal and disease states, such as infertility and germ cell tumors [76].

In TGCTs and other cancers, numerous studies demonstrated that miRNAs can function as oncogenes or tumor suppressors in a tumor-specific manner, suggesting their potential as diagnostic and predictive markers as well as therapeutic targets [76,80-84]. Voorhoeve et al. [74] identified miR-372 and miR-373 (miR-371-373 cluster) as oncogenes in TGCTs. These miRNAs induced proliferation and tumorigenesis of primary human cells by neutralizing tumor protein p53 signaling probably through direct inhibition of large tumor suppressor kinase 2 (LATS2) expression, a protein associated with the Ras pathway [74]. In addition, a high-throughput screen of 156 miRNAs by qPCR in a series of samples (type II and III GCTs, embryonal carcinoma-derived cell lines, and normal testis) showed that those miRNAs could be used to discriminate type II and III GCTs, as well as the histological components of type II GCTs. They also confirmed the role of the miR-371-373 cluster in tumor development [85].

Infertile men are at higher risk to develop TGCTs compared to fertile men, and miRNAs have been implicated to control the pathways connecting male infertility and tumor development [86,87]. The expression of miR-383 is downregulated

in the testes of infertile men with MA. Lian et al. [87] showed that the downregulation of miR-383 and upregulation of interferon regulatory factor 1 (IRF1) in germ cells from patients with MA was associated with increased proliferative activity of cells. On the other hand, the overexpression of miR-383 in NTERA-2 testicular embryonal carcinoma cells led to the suppression of proliferation, arrest in G1-phase and induction of apoptosis. IRF1 was indicated to have a pro-mitogenic role in spermatogonia and early spermatocytes and its expression is normally downregulated by miR-383. Thus, aberrant testicular expression of miR-383 and its effects on IRF1 and IRF1 target, cell cycle-related, genes suggest a possible connection between male infertility and TGCT [87].

It is also noteworthy to mention significant findings regarding miRNA role in prostate cancer (PCa), especially the diagnostic and prognostic value of circulatory miRNAs in PCa patients [88]. Numerous studies identified individual miRNAs that are up or downregulated in PCa patients compared to healthy controls, and notably, miR-141, miR-200b and miR-375 were identified to correlate with disease severity and could potentially be used to identify PCa patients with previously undetectable micrometastases [88]. A complex interaction between miRNA expression, androgen signaling and key pathways in prostate tumorigenesis was suggested [89]. While some miRNAs, such as miR-125b [90], miR-21, miR-32, miR-27a and miR-141, were shown to be regulated by androgen-receptor (AR) mediated signaling, others (e.g., miR-221, miR-222, miR-331-3p and miR-let7c) were suggested to modulate the AR signaling pathway function [89].

Todorova et al. [91] investigated the switch of miRNAs function (and related transcription factors) from initial tumor-suppressor to pro-oncogenic activity, due to genomic instability and rearrangement events in cancer cells. They used several PCa cell lines as models for lymph-node and bone marrow metastases and analyzed the relationship between miR-204, known for its dualistic role in cancer, and the transcriptional factors c-MYB (v-myb avian myeloblastosis viral oncogene homolog), RUNX2 (runt-related transcription factor 2) and ETS1 (v-ets erythroblastosis virus E26 oncogene homolog 1) involved in malignant lineage development, epithelial–mesenchymal transition (EMT) and metastasis. They showed dualistic effects of miR-204 on the expression of transcription factors in PCa cells, acting either as a tumor-suppressor (on c-MYB) or as an oncogene (on ETS1), which was related to DNA methylation patterns and the presence of *TMPRSS2-ERG* gene fusion in PCa cells (a fusion between androgen-sensitive promoter of the transmembrane protease serine 2 gene [*TMPRSS2*] and the v-ets erythroblastosis virus E26 oncogene homolog gene [*ERG*] belonging to ETS family transcription factors) [91]. Furthermore, these authors showed that miR-204 upregulates AR and downregulates TMPRSS2/ERG by directly modulating the methylation of their promoters and through related transcription factors, which prevents TMPRSS2/ERG overexpression in PCa [92]. Hsu et al. [93] described the oncogenic function of another miRNA, miR-18a, in PCa. miR-18a was significantly upregulated in their clinical PCa specimens and cancer cell lines, compared to normal controls. Their experiments with PCa cell lines and mouse model indicated that miR-18a promotes the tumorigenicity of PCa cells, by suppressing the serine/threonine-protein kinase 4 (STK4)-mediated AKT apoptosis pathway [93]. Overall, these studies indicate that modulating disrupted miRNA/target protein interactions in cancer patients may be a promising novel therapeutic strategy.

Depending on miRNA function/effects in cells, two main modes of their targeted modulation in cancer and other diseases have been proposed; i.e., either to restore/induce tumor-suppressive miRNAs or to inhibit those with oncogenic function [94]. Nevertheless, several issues must be overcome before exploring the full potential of miRNAs in the treatment of disease. These, among others, include the delivery and high expense of artificially modified nucleic acids. For example, frequent injections of miRNAs may not be a suitable option for the treatment of sub/infertility in otherwise healthy individuals, although it could be used in the treatment of disease. Also, delivering specific miRNAs to targeted tissues remains a challenge, most importantly because these miRNAs may have different functions in different tissues [76].

CONCLUSION AND FUTURE PERSPECTIVE

Understanding regulatory mechanisms involved in spermatogenesis is of utmost importance for developing effective therapeutic strategies to treat male infertility and diseases of male reproductive system. Over the past decade, a growing number of studies have demonstrated that non-protein coding RNAs, such as miRNAs, play an essential role in the regulation of biological processes, including spermatogenesis. Experimental evidence shows that aberrant expression of these miRNAs affects spermatogenesis at multiple stages and in different cell types, most often resulting in infertility. In more severe cases, dysregulation of miRNAs leads to cancer. Thus, miRNAs have enormous potential to be used as diagnostic and prognostic markers as well as therapeutic targets in male infertility and male reproductive system diseases. To exploit this potential fully, we need a better understanding of miRNA-mediated regulation of spermatogenesis, including the characterization of yet unidentified miRNAs and related regulatory mechanisms. Continuous technological advances, especially in omics technologies, may provide the necessary means to accomplish these challenging tasks.

ACKNOWLEDGMENTS

The authors are thankful to the Vice Chancellor, University of the Punjab, Lahore, Pakistan for providing financial support for the accomplishment of this review.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

REFERENCES

[1] Lian J, Tian H, Liu L, Zhang XS, Li WQ, Deng YM, et al. Downregulation of microRNA-383 is associated with male infertility and promotes testicular embryonal carcinoma cell proliferation by targeting IRF1. Cell Death Dis 2010;1:e94. https://doi.org/10.1038/cddis.2010.70.

[2] Hung AJ, King P, Schlegel PN. Uniform testicular maturation arrest: A unique subset of men with nonobstructive azoospermia. J Urol 2007;178(2):608-12. https://doi.org/10.1016/j.juro.2007.03.125.

[3] Ibtisham F, Wu J, Xiao M, An L, Banker Z, Nawab A, et al. Progress and future prospect of in vitro spermatogenesis. Oncotarget 2017;8(39):66709-27. https://doi.org/10.18632/oncotarget.19640.

[4] Papaioannou MD, Nef S. microRNAs in the testis: Building up male fertility. J Androl 2010;31(1):26-33. https://doi.org/10.2164/jandrol.109.008128.

[5] Lin WW, Lamb DJ, Lipshultz LI, Kim ED. Demonstration of testicular apoptosis in human male infertility states using a DNA laddering technique. Int Urol Nephrol 1999;31(3):361-70. https://doi.org/10.1023/A:1007130320700.

[6] Steger K, Aleithe I, Behre H, Bergmann M. The proliferation of spermatogonia in normal and pathological human seminiferous epithelium: An immunohistochemical study using monoclonal antibodies against Ki-67 protein and proliferating cell nuclear antigen. Mol Hum Reprod 1998;4(3):227-33. https://doi.org/10.1093/molehr/4.3.227.

[7] Maymon BB-S, Yogev L, Yavetz H, Lifschitz-Mercer B, Schreiber L, Kleiman SE, et al. Spermatogonial proliferation patterns in men with azoospermia of different etiologies. Fertil Steril 2003;80(5):1175-80. https://doi.org/10.1016/S0015-0282(03)02161-7.

[8] Gonsalves J, Sun F, Schlegel PN, Turek PJ, Hopps CV, Greene C, et al. Defective recombination in infertile men. Hum Mol Genet 2004;13(22):2875-83. https://doi.org/10.1093/hmg/ddh302.

[9] Sun F, Kozak G, Scott S, Trpkov K, Ko E, Mikhaail-Philips M, et al. Meiotic defects in a man with non-obstructive azoospermia: Case report. Hum Reprod 2004;19(8):1770-3. https://doi.org/10.1093/humrep/deh335.

[10] Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, et al. The transcriptional landscape of the mammalian genome. Science 2005;309(5740):1559-63. https://doi.org/10.1126/science.1112014.

[11] Ambros V. The functions of animal microRNAs. Nature 2004;431(7006):350-5. https://doi.org/10.1038/nature02871.

[12] Bartel DP. MicroRNAs: Target recognition and regulatory functions. Cell 2009;136(2):215-33. https://doi.org/10.1016/j.cell.2009.01.002.

[13] Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. Nat Rev Genet 2007;8(2):93-103. https://doi.org/10.1038/nrg1990.

[14] Kusenda B, Mraz M, Mayer J, Pospisilova S. MicroRNA biogenesis, functionality and cancer relevance. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2006;150(2):205-15. https://doi.org/10.5507/bp.2006.029.

[15] Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;75(5):843-54. https://doi.org/10.1016/0092-8674(93)90529-Y.

[16] Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 2006;34:D140-4. https://doi.org/10.1093/nar/gkj112.

[17] Berezikov E, Cuppen E, Plasterk RH. Approaches to microRNA discovery. Nat Genet 2006;38:S2-7. https://doi.org/10.1038/ng1794.

[18] Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007;129(7):1401-14. https://doi.org/10.1016/j.cell.2007.04.040.

[19] Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009;19(1):92-105. https://doi.org/10.1101/gr.082701.108.

[20] Baskerville S, Bartel DP. Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. RNA 2005;11(3):241-7. https://doi.org/10.1261/rna.7240905.

[21] Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010;11(9):597-610. https://doi.org/10.1038/nrg2843.

[22] Le Bot N. miRNAs and cell-cycle control in ESCs. Nat Cell Biol 2012;14:658. https://doi.org/10.1038/ncb2544.

[23] Cheng AM, Byrom MW, Shelton J, Ford LP. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Res 2005;33(4):1290-7. https://doi.org/10.1093/nar/gki200.

[24] Dennis LM. MicroRNAs in early embryonic development: Dissecting the role of miR-290 through miR-295 in the mouse. Massachusetts, MA, USA: Massachusetts Institute of Technology; 2008.

[25] Latronico MV, Condorelli G. MicroRNAs and cardiac pathology. Nat Rev Cardiol 2009;6(6):419-29. https://doi.org/10.1038/nrcardio.2009.56.

[26] Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 2006;6(4):259-69. https://doi.org/10.1038/nrc1840.

[27] Hansen T, Olsen L, Lindow M, Jakobsen KD, Ullum H, Jonsson E, et al. Brain expressed microRNAs implicated in schizophrenia etiology. PloS One 2007;2(9):e873. https://doi.org/10.1371/journal.pone.0000873.

[28] Li JY, Yong TY, Michael MZ, Gleadle JM. Review: The role of microRNAs in kidney disease. Nephrology (Carlton) 2010;15(6):599-608. https://doi.org/10.1111/j.1440-1797.2010.01363.x.

[29] Sonkoly E, Wei T, Janson PC, Saaf A, Lundeberg L, Tengvall-Linder M, et al. MicroRNAs: Novel regulators involved in the pathogenesis of psoriasis. PloS One 2007;2(7):e610. https://doi.org/10.1371/journal.pone.0000610.

[30] Eisenberg I, Eran A, Nishino I, Moggio M, Lamperti C, Amato AA, et al. Distinctive patterns of microRNA expression in primary muscular disorders. Proc Natl Acad Sci U S A 2007;104(43):17016-21. https://doi.org/10.1073/pnas.0708115104.

[31] Kantharidis P, Wang B, Carew RM, Lan HY. Diabetes complications: The microRNA perspective. Diabetes 2011;60(7):1832-7. https://doi.org/10.2337/db11-0082.

[32] Selbach M, Schwanhausser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. Nature 2008;455(7209):58-63. https://doi.org/10.1038/nature07228.

[33] Kedde M, Agami R. Interplay between microRNAs and RNA-binding proteins determines developmental processes. Cell Cycle

2008;7(7):899-903.
https://doi.org/10.4161/cc.7.7.5644.

[34] Sendler E, Johnson GD, Mao S, Goodrich RJ, Diamond MP, Hauser R, et al. Stability, delivery and functions of human sperm RNAs at fertilization. Nucleic Acids Res 2013;41(7):4104-17.
https://doi.org/10.1093/nar/gkt132.

[35] Pratt SL, Calcatera SM. Expression of microRNA in male reproductive tissues and their role in male fertility. Reprod Fertil Dev 2016;29(1):24-31.
https://doi.org/10.1071/RD16293.

[36] Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, et al. Dicer is essential for mouse development. Nat Genet 2003;35(3):215-7.
https://doi.org/10.1038/ng1253.

[37] Björkgren I, Gylling H, Turunen H, Huhtaniemi I, Strauss L, Poutanen M, et al. Imbalanced lipid homeostasis in the conditional Dicer1 knockout mouse epididymis causes instability of the sperm membrane. FASEB J 2015;29(2):433-42.
https://doi.org/10.1096/fj.14-259382.

[38] Björkgren I, Saastamoinen L, Krutskikh A, Huhtaniemi I, Poutanen M, Sipila P. Dicer1 ablation in the mouse epididymis causes dedifferentiation of the epithelium and imbalance in sex steroid signaling. PloS One 2012;7(6):e38457.
https://doi.org/10.1371/journal.pone.0038457.

[39] Korhonen HM, Meikar O, Yadav RP, Papaioannou MD, Romero Y, Da Ros M, et al. Dicer is required for haploid male germ cell differentiation in mice. PloS One 2011;6(9):e24821.
https://doi.org/10.1371/journal.pone.0024821.

[40] Papaioannou MD, Lagarrigue Me, Vejnar CE, Rolland AD, Kuhne F, Aubry F, et al. Loss of Dicer in Sertoli cells has a major impact on the testicular proteome of mice. Mol Cell Proteomics 2011;10(4):M900587-MCP200.
https://doi.org/10.1074/mcp.M900587-MCP200.

[41] Zimmermann C, Romero Y, Warnefors M, Bilican A, Borel C, Smith LB, et al. Germ cell-specific targeting of DICER or DGCR8 reveals a novel role for endo-siRNAs in the progression of mammalian spermatogenesis and male fertility. PloS One 2014;9(9):e107023.
https://doi.org/10.1371/journal.pone.0107023.

[42] Romero Y, Meikar O, Papaioannou MD, Conne B, Grey C, Weier M, et al. Dicer1 depletion in male germ cells leads to infertility due to cumulative meiotic and spermiogenic defects. PLoS One 2011;6(10):e25241.
https://doi.org/10.1371/journal.pone.0025241.

[43] Hayashi K, de Sousa Lopes SMC, Kaneda M, Tang F, Hajkova P, Lao K, et al. MicroRNA biogenesis is required for mouse primordial germ cell development and spermatogenesis. PloS One 2008;3(3):e1738.
https://doi.org/10.1371/journal.pone.0001738.

[44] Maatouk DM, Loveland KL, McManus MT, Moore K, Harfe BD. Dicer1 is required for differentiation of the mouse male germline. Biol Reprod 2008;79(4):696-703.
https://doi.org/10.1095/biolreprod.108.067827.

[45] Papaioannou MD, Pitetti JL, Ro S, Park C, Aubry F, Schaad O, et al. Sertoli cell Dicer is essential for spermatogenesis in mice. Dev Biol 2009;326(1):250-9.
https://doi.org/10.1016/j.ydbio.2008.11.011.

[46] Hasuwa H, Ueda J, Ikawa M, Okabe M. miR-200b and miR-429 function in mouse ovulation and are essential for female fertility. Science 2013;341(6141):71-3.
https://doi.org/10.1126/science.1237999.

[47] Comazzetto S, Di Giacomo M, Rasmussen KD, Much C, Azzi C, Morgan M, et al. Oligoasthenoteratozoospermia and infertility in mice deficient for miR-34b/c and miR-449 loci. PloS Genet 2014;10(10):e1004597.
https://doi.org/10.1371/journal.pgen.1004597.

[48] Wu J, Bao J, Kim M, Yuan S, Tang C, Zheng H, et al. Two miRNA clusters, miR-34b/c and miR-449, are essential for normal brain development, motile ciliogenesis, and spermatogenesis. Proc Natl Acad Sci U S A 2014;111(28):E2851-7.
https://doi.org/10.1073/pnas.1407777111.

[49] Salas-Huetos A, Blanco J, Vidal F, Mercader JM, Garrido N, Anton E. New insights into the expression profile and function of micro-ribonucleic acid in human spermatozoa. Fertil Steril 2014;102(1):213-22.
https://doi.org/10.1016/j.fertnstert.2014.03.040.

[50] Lian J, Zhang X, Tian H, Liang N, Wang Y, Liang C, et al. Altered microRNA expression in patients with non-obstructive azoospermia. Reprod Biol Endocrinol 2009;7:13.
https://doi.org/10.1186/1477-7827-7-13.

[51] Abu-Halima M, Backes C, Leidinger P, Keller A, Lubbad AM, Hammadeh M, et al. MicroRNA expression profiles in human testicular tissues of infertile men with different histopathologic patterns. Fertil Steril 2014;101(1):78-86.e2.
https://doi.org/10.1016/j.fertnstert.2013.09.009.

[52] Wu W, Qin Y, Li Z, Dong J, Dai J, Lu C, et al. Genome-wide microRNA expression profiling in idiopathic non-obstructive azoospermia: Significant up-regulation of miR-141, miR-429 and miR-7-1-3p. Hum Reprod 2013;28(7):1827-36.
https://doi.org/10.1093/humrep/det099.

[53] Abu-Halima M, Hammadeh M, Schmitt J, Leidinger P, Keller A, Meese E, et al. Altered microRNA expression profiles of human spermatozoa in patients with different spermatogenic impairments. Fertil Steril 2013;99(5):1249-55.e16.
https://doi.org/10.1016/j.fertnstert.2012.11.054.

[54] Abu-Halima M, Hammadeh M, Backes C, Fischer U, Leidinger P, Lubbad AM, et al. Panel of five microRNAs as potential biomarkers for the diagnosis and assessment of male infertility. Fertil Steril 2014;102(4):989-97.e1.
https://doi.org/10.1016/j.fertnstert.2014.07.001.

[55] Salas-Huetos A, Blanco J, Vidal F, Godo A, Grossmann M, Pons MC, et al. Spermatozoa from patients with seminal alterations exhibit a differential micro-ribonucleic acid profile. Fertil Steril 2015;104(3):591-601.
https://doi.org/10.1016/j.fertnstert.2015.06.015.

[56] Abu-Halima M, Ludwig N, Hart M, Leidinger P, Backes C, Keller A, et al. Altered micro-ribonucleic acid expression profiles of extracellular microvesicles in the seminal plasma of patients with oligoasthenozoospermia. Fertil Steril 2016;106(5):1061-9.e3.
https://doi.org/10.1016/j.fertnstert.2016.06.030.

[57] Wu W, Hu Z, Qin Y, Dong J, Dai J, Lu C, et al. Seminal plasma microRNAs: Potential biomarkers for spermatogenesis status. Mol Hum Reprod 2012;18(10):489-97.
https://doi.org/10.1093/molehr/gas022.

[58] Kotaja N. MicroRNAs and spermatogenesis. Fertil Steril 2014;101(6):1552-62.
https://doi.org/10.1016/j.fertnstert.2014.04.025.

[59] Wang C, Yang C, Chen X, Yao B, Yang C, Zhu C, et al. Altered profile of seminal plasma microRNAs in the molecular diagnosis of male infertility. Clin Chem 2011;57(12):1722-31.
https://doi.org/10.1373/clinchem.2011.169714.

[60] Zhu H, Wu H, Liu X, Evans BR, Medina DJ, Liu CG, et al. Role of MicroRNA miR-27a and miR-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. Biochem Pharmacol 2008;76(5):582-8.
https://doi.org/10.1016/j.bcp.2008.06.007.

[61] Zhou JH, Zhou QZ, Yang JK, Lyu XM, Bian J, Guo WB, et al. MicroRNA-27a-mediated repression of cysteine-rich secretory protein 2 translation in asthenoteratozoospermic patients. Asian J Androl 2017;19(5):591-5.
https://doi.org/10.4103/1008-682X.185001.

[62] Safarinejad MR, Shafiei N, Safarinejad S. Association of polymorphisms in the estrogen receptors alpha, and beta (ESR1, ESR2) with the occurrence of male infertility and semen parameters. J Steroid Biochem Mol Biol 2010;122(4):193-203.
https://doi.org/10.1016/j.jsbmb.2010.06.011.

[63] Carreau S, Genissel C, Bilinska B, Levallet J. Sources of oestrogen in the testis and reproductive tract of the male. Int J Androl 1999;22(4):211-23.
https://doi.org/10.1046/j.1365-2605.1999.00172.x.

[64] Abhari A, Zarghami N, Shahnazi V, Barzegar A, Farzadi L, Karami H, et al. Significance of microRNA targeted estrogen

receptor in male fertility. Iran J Basic Med Sci 2014;17(2):81-6.
[65] Li Z, Zheng Z, Ruan J, Li Z, Zhuang X, Tzeng CM. Integrated analysis miRNA and mRNA profiling in patients with severe oligozoospermia reveals miR-34c-3p downregulates PLCXD3 expression. Oncotarget 2016;7(33):52781-96. https://doi.org/10.18632/oncotarget.10947.
[66] Tang D, Huang Y, Liu W, Zhang X. Up-regulation of microRNA-210 is associated with spermatogenesis by targeting IGF2 in male infertility. Med Sci Monit 2016;22:2905-10. https://doi.org/10.12659/MSM.897340.
[67] Li H, Huang S, Guo C, Guan H, Xiong C. Cell-free seminal mRNA and microRNA exist in different forms. PloS One 2012;7(4):e34566. https://doi.org/10.1371/journal.pone.0034566.
[68] Belleannée C, Légaré C, Calvo z, Thimon V, Sullivan R. microRNA signature is altered in both human epididymis and seminal microvesicles following vasectomy. Hum Reprod 2013;28(6):1455-67. https://doi.org/10.1093/humrep/det088.
[69] Li J, Liu Y, Dong D, Zhang Z. Evolution of an X-linked primate-specific micro RNA cluster. Mol Biol Evol 2010;27(3):671-83. https://doi.org/10.1093/molbev/msp284.
[70] Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007;129(7):1401-14. https://doi.org/10.1016/j.cell.2007.04.040.
[71] Belleannée C. Extracellular microRNAs from the epididymis as potential mediators of cell-to-cell communication. Asian J Androl 2015;17(5):730-6. https://doi.org/10.4103/1008-682X.155532.
[72] Yuan S, Tang C, Zhang Y, Wu J, Bao J, Zheng H, et al. mir-34b/c and mir-449a/b/c are required for spermatogenesis, but not for the first cleavage division in mice. Biol Open 2015;4(2):212-23. https://doi.org/10.1242/bio.201410959.
[73] Braun RE. Post-transcriptional control of gene expression during spermatogenesis. Semin Cell Dev Biol 1998;9(4):483-9. https://doi.org/10.1006/scdb.1998.0226.
[74] Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, Nagel R, et al. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell 2006;124(6):1169-81. https://doi.org/10.1016/j.cell.2006.02.037.
[75] Rajender S, Meador C, Agarwal A. Small RNA in spermatogenesis and male infertility. Front Biosci (Schol Ed) 2012;4:1266-74.
[76] McIver SC, Roman SD, Nixon B, McLaughlin EA. miRNA and mammalian male germ cells. Hum Reprod Update 2012;18(1):44-59. https://doi.org/10.1093/humupd/dmr041.
[77] Bjork JK, Sandqvist A, Elsing AN, Kotaja N, Sistonen L. miR-18, a member of Oncomir-1, targets heat shock transcription factor 2 in spermatogenesis. Development 2010;137(19):3177-84. https://doi.org/10.1242/dev.050955.
[78] Yu Z, Raabe T, Hecht NB. MicroRNA Mirn122a reduces expression of the posttranscriptionally regulated germ cell transition protein 2 (Tnp2) messenger RNA (mRNA) by mRNA cleavage. Biol Reprod 2005;73(3):427-33. https://doi.org/10.1095/biolreprod.105.040998.
[79] Bouhallier F, Allioli N, Lavial F, Chalmel F, Perrard MH, Durand P, et al. Role of miR-34c microRNA in the late steps of spermatogenesis. RNA 2010;16(4):720-31. https://doi.org/10.1261/rna.1963810.
[80] Rounge TB, Furu K, Skotheim RI, Haugen TB, Grotmol T, Enerly E. Profiling of the small RNA populations in human testicular germ cell tumors shows global loss of piRNAs. Mol Cancer 2015;14:153. https://doi.org/10.1186/s12943-015-0411-4.
[81] Garzon R, Calin GA, Croce CM. MicroRNAs in cancer. Annu Rev Med 2009;60:167-79. https://doi.org/10.1146/annurev.med.59.053006.104707.
[82] Palmer RD, Murray MJ, Saini HK, Van Dongen S, Abreu-Goodger C, Muralidhar B, et al. Malignant germ cell tumors display common microRNA profiles resulting in global changes in expression of messenger RNA targets. Cancer Res 2010;70(7):2911-23. https://doi.org/10.1158/0008-5472.CAN-09-3301.
[83] Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6(11):857-66. https://doi.org/10.1038/nrc1997.
[84] Costa AL, Lobo J, Jeronimo C, Henrique R. The epigenetics of testicular germ cell tumors: Looking for novel disease biomarkers. Epigenomics 2017;9(2):155-69. https://doi.org/10.2217/epi-2016-0081.
[85] Gillis AJM, Stoop HJ, Hersmus R, Oosterhuis JW, Sun Y, Chen C, et al. High-throughput microRNAome analysis in human germ cell tumours. J Pathol 2007;213(3):319-28. https://doi.org/10.1002/path.2230.
[86] Bezan A, Gerger A, Pichler M. MicroRNAs in testicular cancer: Implications for pathogenesis, diagnosis, prognosis and therapy. Anticancer Res 2014;34(6):2709-13.
[87] Lian J, Tian H, Liu L, Zhang XS, Li WQ, Deng YM, et al. Downregulation of microRNA-383 is associated with male infertility and promotes testicular embryonal carcinoma cell proliferation by targeting IRF1. Cell Death Dis 2010;1:e94. https://doi.org/10.1038/cddis.2010.70.
[88] Sita-Lumsden A, Dart DA, Waxman J, Bevan CL. Circulating microRNAs as potential new biomarkers for prostate cancer. Br J Cancer 2013;108(10):1925-30. https://doi.org/10.1038/bjc.2013.192.
[89] Jackson BL, Grabowska A, Ratan HL. MicroRNA in prostate cancer: Functional importance and potential as circulating biomarkers. BMC Cancer 2014;14:930. https://doi.org/10.1186/1471-2407-14-930.
[90] Shi XB, Xue L, Ma AH, Tepper CG, Kung HJ, White RW. miR-125b promotes growth of prostate cancer xenograft tumor through targeting pro-apoptotic genes. Prostate 2011;71(5):538-49. https://doi.org/10.1002/pros.21270.
[91] Todorova K, Metodiev MV, Metodieva G, Zasheva D, Mincheff M, Hayrabedyan S. miR-204 is dysregulated in metastatic prostate cancer in vitro. Mol Carcinog 2016;55(2):131-47. https://doi.org/10.1002/mc.22263.
[92] Todorova K, Metodiev MV, Metodieva G, Mincheff M, Fernandez N, Hayrabedyan S. Micro-RNA-204 participates in TMPRSS2/ERG regulation and androgen receptor reprogramming in prostate cancer. Horm Cancer 2017;8(1):28-48. https://doi.org/10.1007/s12672-016-0279-9.
[93] Hsu TI, Hsu CH, Lee KH, Lin JT, Chen CS, Chang KC, et al. MicroRNA-18a is elevated in prostate cancer and promotes tumorigenesis through suppressing STK4 in vitro and in vivo. Oncogenesis 2014;3:e99. https://doi.org/10.1038/oncsis.2014.12.
[94] Li C, Feng Y, Coukos G, Zhang L. Therapeutic microRNA strategies in human cancer. AAPS J 2009;11(4):747-57. https://doi.org/10.1208/s12248-009-9145-9.
[95] Xie R, Lin X, Du T, Xu K, Shen H, Wei F, et al. Targeted disruption of miR-17-92 impairs mouse spermatogenesis by activating mTOR signaling pathway. Medicine (Baltimore) 2016;95(7):e2713. https://doi.org/10.1097/MD.0000000000002713.
[96] Tong MH, Mitchell DA, McGowan SD, Evanoff R, Griswold MD. Two miRNA clusters, Mir-17-92 (Mirc1) and Mir-106b-25 (Mirc3), are involved in the regulation of spermatogonial differentiation in mice. Biol Reprod 2012;86(3):72. https://doi.org/10.1095/biolreprod.111.096313.
[97] Medeiros LA, Dennis LM, Gill ME, Houbaviy H, Markoulaki S, Fu D, et al. Mir-290-295 deficiency in mice results in partially penetrant embryonic lethality and germ cell defects. Proc Natl Acad Sci U S A 2011;108(34):14163-8. https://doi.org/10.1073/pnas.1111241108.
[98] Huszar JM, Payne CJ. MicroRNA 146 (Mir146) modulates spermatogonial differentiation by retinoic acid in mice. Biol Reprod 2013;88(1):15. https://doi.org/10.1095/biolreprod.112.103747.
[99] Yang QE, Racicot KE, Kaucher AV, Oatley MJ, Oatley JM. MicroRNAs 221 and 222 regulate the undifferentiated state in mammalian male germ cells. Development 2013;140(2):280-90. https://doi.org/10.1242/dev.087403.
[100] Niu Z, Goodyear SM, Rao S, Wu X, Tobias JW, Avarbock MR, et al.

MicroRNA-21 regulates the self-renewal of mouse spermatogonial stem cells. Proc Natl Acad Sci U S A 2011;108(31):12740-5. https://doi.org/10.1073/pnas.1109987108.

[101] Moritoki Y, Hayashi Y, Mizuno K, Kamisawa H, Nishio H, Kurokawa S, et al. Expression profiling of microRNA in cryptorchid testes: miR-135a contributes to the maintenance of spermatogonial stem cells by regulating FoxO1. J Urol 2014;191(4):1174-80. https://doi.org/10.1016/j.juro.2013.10.137.

[102] Bao J, Li D, Wang L, Wu J, Hu Y, Wang Z, et al. MicroRNA-449 and microRNA-34b/c function redundantly in murine testes by targeting E2F transcription factor-retinoblastoma protein (E2F-pRb) pathway. J Biol Chem 2012;287(26):21686-98. https://doi.org/10.1074/jbc.M111.328054.

[103] Dai L, Tsai-Morris CH, Sato H, Villar J, Kang JH, Zhang J, et al. Testis-specific miRNA-469 up-regulated in gonadotropin-regulated testicular RNA helicase (GRTH/DDX25)-null mice silences transition protein 2 and protamine 2 messages at sites within coding region: Implications of its role in germ cell development. J Biol Chem 2011;286(52):44306-18. https://doi.org/10.1074/jbc.M111.282756.

[104] Yuan S, Tang C, Zhang Y, Wu J, Bao J, Zheng H, et al. mir-34b/c and mir-449a/b/c are required for spermatogenesis, but not for the first cleavage division in mice. Biol Open 2015;4(2):212-23. https://doi.org/10.1242/bio.201410959.