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REVIEW

The microRNA-mediated apoptotic signaling axis in male reproduction: a possible and targetable culprit in male infertility

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Abstract Recently, infertility has emerged as a significant and prevalent public health concern warranting considerable attention. Apoptosis, recognized as programmed cell death, constitutes a crucial process essential for the maintenance of normal spermatogenesis. Multiple investigations have illustrated that the dysregulated apoptosis of reproductive cells, encompassing spermatogonial stem cells, Sertoli cells, and Leydig cells, serves as a causative factor in male infertility. MicroRNAs represent a class of small RNA molecules that exert negative regulatory control over gene expression using direct interaction with messenger RNA transcripts. Previous studies have established that aberrant expression of miRNAs induces apoptosis in reproductive tissues, correlating with reproductive dysfunctions and infertility. In this review, we offer a comprehensive overview of miRNAs and their respective target genes implicated in the apoptotic process. As well, miRNAs are involved in multiple apoptotic signaling pathways, namely the PI3K/AKT, NOTCH, Wnt/β-catenin, and mTOR signaling cascades, exerting both negative and positive effects. We additionally elucidate the significant functions played by lncRNAs and circular RNAs as competing endogenous RNAs in the process of apoptosis within reproductive cells. We further illustrate that external factors, including silica nanoparticles, Cyclosporine A, and smoking, induce dysregulation of miRNAs, resulting in apoptosis within reproductive cells and subsequent male reproductive toxicity.

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Further, we discuss the implication of heat stress, hypoxia, and diabetes in reproductive cell apoptosis induced by miRNA dysregulation in male infertility. Finally, we demonstrate that the modulation of miRNAs via traditional and novel medicine could protect reproductive cells from apoptosis and be implemented as a therapeutic approach in male infertility.

Highlights

- A comprehensive overview of miRNAs and their respective target genes implicated in the apoptotic process.
- External factors including silica nanoparticles, Cyclosporine A, and smoking induce dysregulation of miRNAs, resulting in apoptosis within reproductive cells and subsequent male reproductive toxicity.
- Implication of heat stress, hypoxia, and diabetes in reproductive cell apoptosis induced by miRNA dysregulation in male infertility.
- Modulation of miRNAs via traditional and novel medicine could protect reproductive cells from apoptosis and be implemented as a therapeutic approach in male infertility.

Keywords MiRNAs · Apoptosis · Infertility

Introduction

In the past few decades, infertility has emerged as a significant and prevalent public health concern warranting substantial attention. An estimated 50% of cases of infertility stem from male factors, and the causes are complex, involving multiple factors including genetic, environmental, and epigenetic regulation (Sharma et al. 2023; Yan et al. 2021). Male infertility is often associated with dysfunction of spermatogenesis, which subsequently gives rise to issues concerning sperm quality and a diminished sperm count in the seminal fluid. Spermatogenesis is widely recognized as a highly intricate, dynamic, and orchestrated procedure occurring within the epithelium of the seminiferous tubules. The process of spermatogenesis plays a pivotal role in the transmission of genetic material and species maintenance. Spermatogonial stem cells (SSCs), Sertoli cells (SCs), and Leydig cells (LCs) constitute the fundamental cellular components involved in spermatogenesis. The persistence of these cellular entities represents an evolutionary imperative for the organism, given their indispensable role in ensuring the preservation of fertility. However, they could undergo apoptotic cell death when exposed to Male infertility is often associated with dysfunction of spermatogenesis, which subsequently gives rise to issues concerning sperm quality and a diminished sperm count in the seminal fluid (Aslani et al. 2017; Wang et al. 2023). MicroRNAs (miRNAs) represent a class of evolutionarily conserved ribonucleic acids (RNAs), approximately 22–24 nucleotides in length and devoid of protein-coding capability, and have received much attention due to their key role in gene expression regulation (Das et al. 2022). Studies have shown that miRNAs regulate the expression of target genes by targeting the 3' untranslated region (3'UTR) of specific mRNA, and participate in various biological processes such as cell proliferation, differentiation, and apoptosis (Wang et al. 2023). Several miRNAs identified within reproductive cells have been shown to function as significant regulators of apoptosis (He et al. 2021a; Zhou et al. 2020a). MiRNAs may affect the survival and function of male germ cells by regulating the expression of apoptosis-related genes, thus leading to male infertility (Klees et al. 2023; Shi et al. 2024c). Although the important role of miRNAs in male infertility has been widely studied, its specific regulatory mechanisms and clinical applications still need to be further explored. The aim of the current work is therefore to summarise the present knowledge of miRNA action in male reproductive cell physiology concerning apoptosis mechanisms. A thorough comprehension of the miRNA pathways is imperative for the development of prospective therapeutic strategies aimed at mitigating male infertility.

Methodology

The literature on experimental animals, clinical studies, and in vitro studies published up to December 2025 was searched using PubMed, Scopus, and Web of Science databases. Search terms encompassed miRNA, long non-coding RNA (lncRNA), circular RNA (circRNA), apoptosis, signaling pathways, germ cells, spermatogenesis, male infertility, reproductive

toxicity, reproductive dysfunction, drugs, etc. We employed both single-term and multi-term combinations to refine our search and excluded literature not pertinent to this study. Furthermore, we manually examined all references cited in relevant reviews to extract pertinent information conducive to this review.

Main male reproductive cells

The main male reproductive cells, including SSCs, SCs, and LCs, play essential roles in male fertility and spermatogenesis. SSCs, which originate from primordial germ cells during embryogenesis, are located along the seminiferous tubule periphery and are crucial for self-renewal and the continuous production of daughter spermatogonia, which eventually differentiate into spermatozoa (Moghadasi et al. 2023; Yang et al. 2021). Disruption of SSC self-renewal or progenitor emergence can lead to azoospermia, and dysregulation may also contribute to testicular germ cell tumors (Barchi et al., 2023). SCs, which interact with basement membranes and germ cells, are integral to the structural and functional development of the testis, supporting the SSC niche, guiding meiosis and spermiogenesis, and protecting germ cells from immune responses (Amiri et al. 2023; Washburn et al. 2022). Furthermore, LCs, comprising fetal and adult populations, are responsible for synthesizing androgens like testosterone, which regulate spermatogenesis and influence sexual development and behavior (Bhattacharya and Dey 2022). The interactions between LCs, SCs, and germ cells are crucial for maintaining spermatogenesis, as dysfunctional LCs lead to impaired androgen production and disrupted spermatogenesis. Together, these cells form a complex network that ensures proper male reproductive function and fertility.

An overview of apoptosis

Apoptosis, a widely recognized mechanism of controlled cell death, is initiated through the engagement of cell-surface death receptors such as Fas by their ligands, known as the extrinsic pathway, or by the permeabilization of the mitochondrial outer membrane induced by pro-apoptotic proteins, referred to as the intrinsic pathway. The intrinsic pathway is governed and modulated by a subset of proteins classified within the BCL-2 family. These proteins are classified into two groups: pro-apoptotic proteins (including Bid, Bad, Bax, Bcl-10, Bik, Bak, Bim and Hrk), which regulate the release of cytochrome C by modulating mitochondrial membrane permeability, and anti-apoptotic proteins (such as Bcl-XL, Bf-1, Bcl-x, Bcl-w, Bcl-2, B-XS, Bcl-w and BAG), which inhibit cytochrome C release and play a critical role in determining the onset or prevention of apoptosis (Singh et al. 2019). By contrast, the extrinsic pathway operates through the activation of death receptors belonging to the tumor necrosis factor (TNF) receptor gene superfamily. Various death receptors, including TNF-α/TNFR1, FasL/FasR, apoptosis antigen (APO) 2L/DR4, APO3L/DR3 and APO2L/DR5, are present (Mustika et al., 2021; Singh et al. 2022). In the extrinsic pathway, the interaction between Fas and TNF ligands and their corresponding receptors, Fas and TNF receptors, respectively, facilitates binding with their adapter proteins. The association of the adapter protein is correlated with the activation of procaspase-8, concomitant with the self-catalytic activity of caspase-8. Furthermore, the binding and activation processes facilitate the transmission of the death signal via caspase 8 to an execution caspase, thereby inducing apoptosis (Singh et al. 2022; Wajant 2002).

MiRNAs: synthesis and role in apoptosis

MiRNAs are small, noncoding single-stranded RNAs, 21–25 nucleotides in length, found across animals and plants. They regulate gene expression at the post-transcriptional level by binding to complementary sites in the 3′ untranslated region (3′UTR) of target messenger RNAs (mRNAs). Over 1000 human miRNAs have been identified, with more than 30% of the human genome potentially regulated by miRNAs. MiRNA biogenesis begins in the nucleus, where precursor molecules are processed by Drosha and Dicer enzymes to generate mature miRNAs. These are incorporated into the RNA-induced silencing complex, which mediates gene silencing (Song et al. 2020). A single miRNA can target multiple mRNAs, and multiple miRNAs can regulate the same mRNA, forming a complex regulatory network involved in processes like development, proliferation, differentiation, and apoptosis (Ma et al. 2020; Song et al. 2020).

MiRNAs play a crucial role in regulating cell growth and apoptosis (Uzuner et al., 2022). For example, in colorectal cancer cells, miR-148a targets Bcl-2 to potentially activate the intrinsic apoptosis pathway (Elnaggar et al. 2021; Zhang et al. 2011). Additionally, miR-200 can enhance cancer cell sensitivity to apoptosis by targeting FAP-1, an apoptosis inhibitor (Sargolzaei et al. 2020; Schickel et al. 2010). Spermatogenesis is a complex process involving spermatogonia proliferation, differentiation, meiosis and spermatogenesis (Feng et al. 2022; Walker 2022). As important gene expression regulators, miRNAs play a key role in various stages of spermatogenesis. In this review, we focus on the role of miRNAs in regulating apoptosis of male germ cells.

Apoptosis-related miRNAs in reproductive cells: evidence from animal and human studies

MiR-210-3p

MiR-210 is located at locus 11p15.5 on chromosome 11 in humans. MiR-210 manifests in two distinct forms: miR-210-3p, serving as the guide strand, and miR-210-5p, functioning as the passenger strand (Bavelloni et al. 2017). Upregulation of miR-210-3p in seminal plasma has been identified as a potential biomarker for early detection of impaired spermatogenesis in varicocele (VC) patients. The increased expression of miR-210-3p appears to induce spermatogenic cell apoptosis by promoting caspase-3 activation, potentially through a hypoxia-mediated mechanism in VC-induced infertility (Xu et al. 2020b).

MiR-322

MiR-424, the mammalian homolog of rodent miR-322, along with miR-503, represents distinct members of the mammalian-specific miR-15/107 family. These molecules are encoded as a single cluster by H19X located at the human Xq26.3 region, where they play crucial roles in governing both cell division and apoptosis (Wang et al. 2019). Silencing of miR-322 has been shown to correlate with decreased cell viability and a marked increase in apoptosis in GC-2 cells (Che et al. 2019). Furthermore, the downregulation of miR-322 significantly upregulates key apoptotic genes, including Bax and caspases 3, 9, and 8, while concurrently downregulating the anti-apoptotic Bcl-2 gene. Notably, Ddx3x was identified as a direct target of miR-322, mediating its pro-apoptotic effects (Cannarella et al. 2020; Che et al. 2019). Additionally, a notable reduction in miR-322 expression was observed in the seminal plasma of individuals exhibiting a high DNA fragmentation index, a hallmark of male infertility. This reduction in miR-424 (murine homologue miR-322) expression among infertile males likely contributes to the induction of apoptosis in spermatogenic cells and DNA damage in spermatozoa through direct interaction with its target gene, Ddx3x, thus playing a role in the pathogenesis of male infertility (Cannarella et al. 2020; Che et al. 2019; Wang et al. 2020a).

MiR-130a

MiR-130a is located at locus 11q12.1 on chromosome 11 in humans. In mice, miR-130a has been reported to be present in all tissues and is particularly abundant in the testis (Li et al. 2018a). MiR-130a directly targets with the 3'-UTR of the androgen receptor (AR), and its overexpression leads to a significant reduction in AR expression, as observed in both in vitro and in vivo models. Furthermore, mice injected intratesticularly with miR-130a showed impaired spermatogenesis and increased apoptosis among germ cells. Collectively, these findings suggest that miR-130a downregulates AR expression in SCs, subsequently inducing apoptosis in germ cells and disrupting spermatogenesis (Davis et al. 2024; Li et al. 2018a; Wang et al. 2022a).

MiR-100-3p

MiR-100, a member of the miR-100 family, is one of the most evolutionarily conserved miRNA families, located on chromosome 11 at locus 11q24.1 (Fuso et al. 2021; Ye et al. 2020). Elevated expression of miR-100-3p is observed in human SCs, with miR-100-3p mimics exhibiting a mitigating effect on apoptosis in these cells. MiR-100-3p directly targets serum/glucocorticoid-regulated kinase family member 3 (SGK3), and depletion of SGK3 enhanced cell proliferation while decreasing apoptosis in human SCs. Collectively, miR-100-3p functions as a novel epigenetic regulator in human SCs, modulating apoptosis through its targeting of SGK3. The finding

provides a potential therapeutic approach for gene therapy in male infertility (Cassuto et al. 2025; Huang et al. 2024; Liu et al. 2021a).

MiR-362

MiR-362 is located at Xp11.23 on the X chromosome in humans. Silencing miR-362 resulted in the attenuation of apoptosis, facilitated cell progression into the S phase, and upregulated cell cycle-associated genes such as *c-MYC*, *CCND1*, *CDK4*, and *CNNE1* in immature porcine SCs (Shi et al. 2024c). Additionally, miR-362 selectively targets the recQ-mediated genome instability protein 1 (*RMI1*) gene, thereby suppressing *RMI1* protein expression. Notably, the effects of miR-362 inhibition on porcine immature SCs were additionally nullified by siRNA-mediated knock-down of *RMI1*. These findings suggest that miR-362 regulates the fate of immature SCs by modulating *RMI1* expression, promoting cell proliferation while inhibiting apoptosis (Ran et al. 2020).

MiR-26a

The miR-26 family, comprising miR-26b, miR-26a, miR-4465, and miR-1297, comprises a group of highly conserved small RNAs, all sharing identical sequences within their seed region (Li et al. 2021a). Human miR-26a is sheared from two precursors, with miR-26a-1 located at the 3q22.2 locus on chromosome 3 and miR-26a-2 located at the 12q14.1 locus on chromosome 12. MiR-26a has been shown to directly target and downregulate the expression of *PAK2* mRNA in SCs. Furthermore, Upregulation of miR-26a inhibited cell proliferation and promoted apoptosis, effects that closely resembled those observed upon siRNA-mediated knockdown of *PAK2*. Collectively, these findings suggest that miR-26a plays a critical role in modulating SCs function by suppressing proliferation and inducing apoptosis through *PAK2* targeting, thus serving as a potential regulatory factor in spermatogenesis (Alves et al. 2020; Ran et al. 2018; Shi et al. 2024c).

MiR-196a

The miRNA miR-196a encompasses two mature forms bearing identical sequences: miR-196a1 located on chromosome 17q21.32 and miR-196a2 situated on chromosome 12q13.13. MiRNA-196a has been shown to promote the proliferation of immature SCs while inhibiting their apoptosis (Bian et al. 2021). MiR-196a exerts its effects by targeting *RCC2* and *ABCB9*, showing a negative correlation with both the mRNA and protein levels of these genes. Thus, miR-196a regulates the balance between proliferation and apoptosis in immature SCs through the downregulation of *RCC2* and *ABCB9* expression (Chen et al. 2023; Shi et al. 2024c; Zhang et al. 2019).

MiR-34

The miR-34 family encompasses three distinct members, namely miR-34a, miR-34b and miR-34c (Pantos et al. 2021; Welponer et al. 2020). MiR-34a resides within the second exon of a gene situated on chromosome 1p36.22, while miR-34b and miR-34c are harboured by a shared host gene located on chromosome 11q23.1. MiR-34c plays a crucial role in spermatogenesis, being expressed in mouse pachytene spermatocytes and spermatids (Joshi et al. 2023; Shi et al. 2024c). Depletion of miR-34c increases the Bcl-2/Bax ratio, preventing germ cell apoptosis triggered by testosterone deprivation. Conversely, overexpression of miR-34c in GC-2 cells induces apoptosis with a decreased Bcl-2/Bax ratio, while silencing miR-34c results in a reduced apoptotic rate and higher Bcl-2/Bax ratio. Additionally, miR-34c directly targets the 3′UTR of activating transcription factor 1 (ATF1), downregulating its expression and influencing apoptosis. Silencing ATF1 mimics the effects of miR-34c overexpression, indicating a crucial role of ATF1 in miR-34c-induced apoptosis (Barbu et al. 2021; Liang et al. 2012; Vashisht and Gahlay 2020). Moreover, miR-34c targets SMAD family member 7 (*SMAD7)* in immature porcine SCs, inhibiting proliferation and promoting apoptosis, similar to the effects observed when *SMAD7* is silenced by siRNA. The expression of miR-34c increases with age in swine testicular tissue, inversely correlating with *SMAD7* expression (RAN et al. 2019). Additionally, hsa-miR-34b-5p targets inositol 1,4,5-Trisphosphate Receptor Type 1 (*ITPR1*) and is upregulated in non-obstructive azoospermia (NOA) patients. ITPR1 plays a key role in the Ca^{2+}-apoptosis pathway, and its elevated expression is linked to spermatogenesis failure in NOA. The hsa-miR-34b-5p/ITPR1 axis may serve as a regulatory biomarker for human spermatogenesis,

mediating the interplay between Ca^{2+} signaling and apoptosis (Maleki et al. 2023; Shi et al. 2024b).

MiR-31

The miR-31 gene, situated on chromosome band 9p21.3, plays a pivotal role as a mediator of the meiotic process in SSCs. Previous studies have shown that miR-31 inhibits meiosis by targeting Stra8, thereby affecting spermatogenesis (Guo et al. 2024; Wang et al. 2017). Epidermal growth factor (EGF) regulates p21-activated kinase 1 (PAK1), promoting human SSC proliferation and reducing apoptosis (Fu et al. 2018). Recent findings suggest that PAK1 suppress of miR-31-5p, and its depletion leads to elevated PAK1 levels. Overexpression of miR-31-5p mimics inhibits cell proliferation and DNA synthesis while enhancing both early and late apoptosis in human SSCs. Additionally, miR-31-5p directly targets JAZF1, downregulating its expression, which in turn reduces cell proliferation and DNA synthesis, and further promotes apoptosis (Ding et al. 2021; Du et al. 2021). Furthermore, miR-31-5p mimics predominantly decrease cyclin A2 expression in human SSCs, with JAZF1 depletion similarly resulting in reduced cyclin A2 levels. Overall, these findings highlight the role of the PAK1-JAZF1-cyclin A2 pathway in regulating apoptosis, DNA synthesis, and proliferation in human SSCs (Fu et al. 2019).

MiR-1908-3p

Hsa-mir-1908 is situated within the initial intronic region of the fatty acid desaturase 1 gene, positioned on chromosome 11. MiR-1908-3p is highly expressed in human spermatogonia compared to pachytene spermatocytes, and it promotes DNA synthesis and cell proliferation in human SSCs. In addition, miR-1908-3p effectively suppresses both early and late apoptosis in human SSCs. Through direct targeting of Kruppel-like factor 2 (KLF2), miR-1908-3p enhances cell proliferation and DNA synthesis while reducing apoptosis. Notably, silencing KLF2 restores cell proliferation and DNA synthesis, and alleviates the apoptosis induced by the inhibition of miR-1908-3p in human SSCs (Chen et al. 2020; Sharma et al. 2023). These findings suggest that miR-1908-3p supports self-renewal and inhibits apoptosis in human SSCs via modulation of KLF2.

MiR-663a

MiR-663a is located at 20p11.1 on chromosome 20 in humans. MiR-663a is upregulated in human spermatogonia compared to pachytene spermatocytes, promoting cell proliferation and DNA synthesis while inhibiting both early and late apoptosis in SSCs. It directly targets NFIX,a member of the nuclear factor I family, and silencing NFIX mimics the effects of miR-663a, enhancing proliferation and DNA synthesis, and reducing apoptosis. Additionally, miR-663a and NFIX silencing both increase cyclins A2, B1, and E1, while miR-663a inhibition decreases them. Knockdown of these cyclins reduces SSC proliferation. In summary, miR-663a promotes SSC self-renewal and suppresses apoptosis through NFIX and cyclin regulation (Du et al. 2021; Gao et al. 2020b; Zhou et al. 2018).

MiR-22-5p

In the mammalian genome, miR-22 originates from an exon within the lncRNA gene known as the miR-22 host gene, located at locus 17p13.3 on chromosome 17 (Huang and Wang 2014; Lv et al. 2022). MiR-22-5p, elevated in the testicular tissues of cryptorchidism patients, negatively regulates SSC proliferation and promotes apoptosis by targeting EZH2. Overexpression of miR-22-5p reduced SSC marker proteins (GDNF and DAZL), while miR-22-5p knockout had the opposite effect. Luciferase assays confirmed EZH2 as a direct target of miR-22-5p, and its overexpression counteracted the effects of miR-22-5p on SSC proliferation and apoptosis (Lv et al. 2022; Shi et al. 2024c). These findings suggest miR-22-5p regulates SSC self-renewal and apoptosis through EZH2, highlighting its potential as a biomarker for cryptorchidism-related infertility (García-Andrade et al. 2022).

MiR-21

The genomic localization and characterization of the human miR-21 gene are established, and the gene has been mapped to chromosome 17q23.2 (Surina et al. 2021). MiR-21 plays a crucial role in maintaining the SSC population. In cultures enriched with SSCs, transient inhibition of miR-21 led to increased apoptosis of germ cells and a significant reduction in

spermatogenesis colony formation from transplanted cells in recipient mice. Additionally, miR-21 expression is regulated by the transcription factor ETV5, which is essential for SSC self-renewal (Sahoo et al. 2021; Tang et al. 2022). Thus, the ETV5/miR-21 axis is a key regulatory mechanism for SSC maintenance (Niu et al. 2011).

MiR-188-3p

MiR-188 is located at the Xp11.23 site of chromosome X in humans, where miR-188-3p plays a key role in germ cell development, with dysregulated expression contributing to disorders in sperm formation (Wang et al. 2022b). In patients with obstructive azoospermia (OA) and NOA, miR-188-3p expression was significantly lower, while MLH1 (a critical gene involved in the pairing of homologous chromosomes during meiosis) expression was elevated at both the mRNA and protein levels. The histone acetylation of the miR-188-3p promoter was decreased in azoospermia patients, with changes in miR-188-3p expression primarily driven by HDAC1 modulation, not HDAC2. MiR-188-3p regulates MLH1 expression by directly binding to its 3′ UTR. Inhibition of miR-188-3p resulted in increased spermatogenic cell apoptosis, an effect that was counteracted by si-MLH1. These findings suggest that miR-188-3p downregulation, through histone acetylation changes, upregulates MLH1, thereby promoting apoptosis in spermatogenic cells in azoospermia (Gao et al. 2019; Shi et al. 2024c; Song et al. 2017).

MiR-10b

MiR-10b, situated on chromosome 2 within the HOXD gene cluster, plays a pivotal role in various cellular processes ranging from differentiation to apoptosis (Wilson et al. 2024). In vitro, miR-10b expression was elevated in SSCs, promoting their proliferation, while its inhibition led to increased apoptosis. This effect is mediated through the direct targeting of Kruppel-like factor 4 (KLF4). The miR-10b-KLF4 pathway plays a key role in SSC self-renewal and apoptosis, offering potential insights for diagnosing and treating male infertility (Li et al. 2017; Shi et al. 2024c; Xu et al. 2020a).

MiR-30a-5p

Derived from an intronic transcriptional unit located on chromosome 6q.13, miR-30a has been implicated in various biological processes, such as apoptosis and proliferation (Jiang et al. 2018). Recent studies have shown that miR-30a-5p mimics can reduce apoptosis in cryopreserved SSCs. Transfecting SSCs with miR-30a-5p mimics before and during freezing–thawing increased SSC colony viability, number, and size. Additionally, miR-30a-5p mimics decreased BAX expression and increased Bcl-2 expression, protecting SSCs from apoptosis. These results suggest that miR-30a-5p could be a valuable strategy for improving SSC cryopreservation in prepubertal boys with cancer (Khanlari et al. 2021).

MiRNAs associated with male germ cell apoptosis have received much attention in animal and human studies. miRNAs play an important role in the survival and function of male germ cells by regulating the expression of apoptosis-related genes (Fig. 1). Animal experiments have revealed the regulatory networks of miRNAs, while human studies have further validated their potential role in male infertility. Despite some limitations, such as differences between animal models and humans and the complexity of miRNAs, future studies should combine multi-omics techniques and clinical data to overcome these limitations and advance the study and application of miRNAs in male infertility.

MiRNA regulation of apoptotic signaling pathways

MiRNA-mediated regulation of the PI3K/AKT pathway

Phosphatidylinositol-3 kinases (PI3Ks) represent a class of lipid kinases responsible for the phosphorylation of the signaling lipid phosphatidylinositol 4,5-bisphosphate to yield phosphatidylinositol 3,4,5-trisphosphate. Three distinct classes of PI3Ks, namely class I, class II, and class III, have been characterized, each exhibiting specific substrates and unique effectors alongside the shared substrate Akt. AKT, recognized as a significant downstream effector of PI3K, can reduce the levels of pro-apoptotic proteins Bad and Bax while increasing the expression

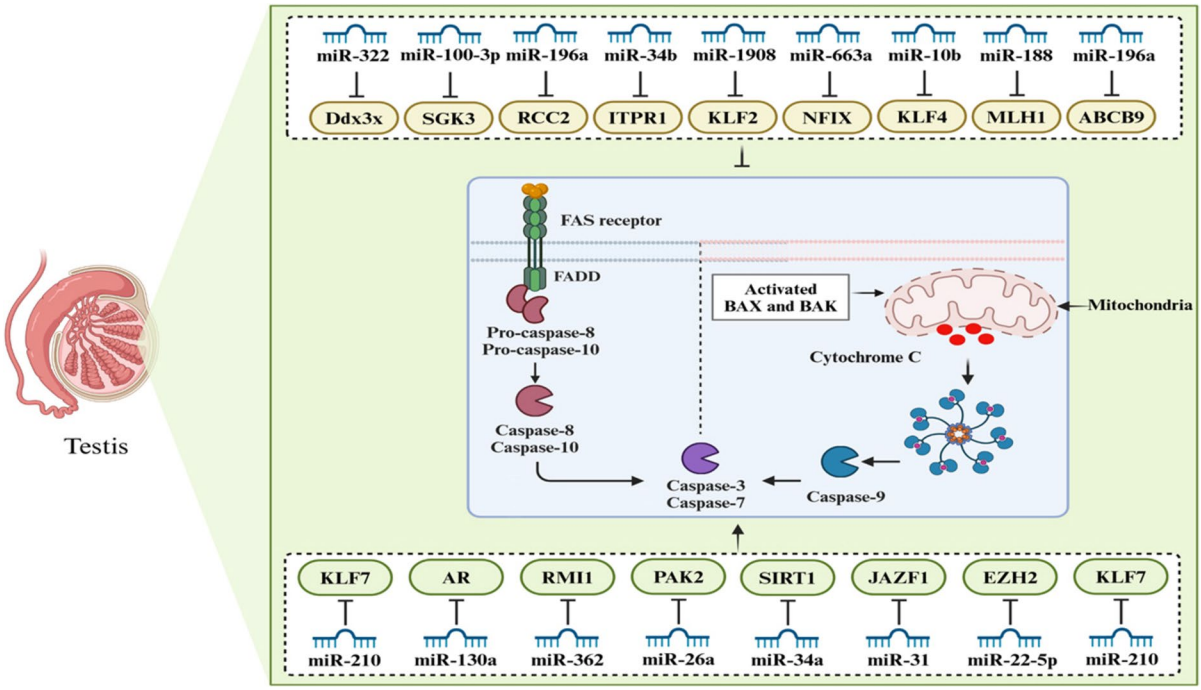

Fig. 1 Representation of key miRNAs implicated in apoptosis regulation within male reproductive cells and their corresponding target genes (the figure was created with BioRender.com)

of anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2), Bcl-xl, and myeloid cell leukemia 1 (Liang et al. 2022; Saddam et al. 2024). Thereby, the PI3K/Akt signaling pathway exerts its influence by promoting cellular proliferation and viability while mitigating growth inhibition and apoptosis (Atif et al. 2015; Ji et al. 2012). Studies have shown that a variety of miRNAs can affect the survival and function of male germline cells by mediating PI3K/AKT signaling axis abnormalities, which may be one of the important mechanisms of male infertility.

The predominant localization of miR-499 within the basement section of seminiferous tubules in prepubertal porcine testicular tissue correlates with its capacity to augment cell proliferation and impede apoptosis upon overexpression, while its inhibition exerts contrasting effects. Studies have found that miR-499 facilitates cellular proliferation and suppresses apoptosis in immature porcine SCs via modulation of the PI3K/AKT pathway, achieved through PTEN gene targeting (Gao et al. 2020a). It has been reported that miR-638 modulates the apoptosis of immature SCs through its interaction with the SPAG1 gene, thereby indirectly suppressing PI3K/AKT pathway activation and contributing to spermatogenesis (Hu et al. 2017). Meanwhile, miR-126 exerts direct targeting of the PIK3R2 gene, a constituent of the PI3K family, and modulates immature porcine SCs apoptosis through the PI3K/AKT signaling cascade (Tang et al. 2021). In addition, miR-125a-5p by targeting RAB3D and modulating the PI3K/AKT pathway miR-125a-5p controls proliferation and apoptosis in SCs (Teng et al. 2021; Yu et al. 2022).

MiRNA-mediated regulation of the NOTCH pathway

Notch receptors, comprising four mammalian family constituents (Notch1-4), are characterized by extensive extracellular EGF-like domains facilitating ligand interaction and intracellular segments facilitating signal transduction (Ghafouri-Fard et al. 2021; Zhou et al. 2022). Following activation, Notch receptors experience a series of metalloprotease tumor necrosis factor-α-converting enzyme and γ-secretase complex proteolytic cleavages, resulting in the liberation of the Notch intracellular domain (NICD) (Sen et al. 2023; Zhou et al. 2022). Subsequently, the NICD undergoes translocation to the nucleus,

where it engages with the DNA-binding protein CSL, thereby modulating gene expression (Sen et al. 2023). The Notch signaling pathway orchestrates cellular apoptosis via either a caspase-dependent or a caspase-independent route (Zhou et al. 2022).

It has been reported that miR-4270 expression is significantly increased in the SCs of patients with s Sertoli cell-only syndrome (SCOS) compared to healthy controls. It was further found that miR-4270 inhibited the NOTCH signaling pathway by targeting the GADD45A gene and regulated proliferation and apoptosis in SCOS patients' SCs, thus providing a new perspective on human support cell development and a potential biomarker for SCOS therapy (Wang et al. 2020b). In addition, a study analyzed seminal plasma miRNA expression in patients with different pathologic types of NOA (SCOS and SA) versus normal fertile men. It was found that miR-34c-5p was significantly lower in NOA patients than in normal fertile controls, and the decrease was more obvious in SCOS patients (Zhang et al. 2021). It has been reported that miR-34c can negatively regulate the Notch signaling pathway, and Notch 1 is a direct target of miR-34c (Luo et al. 2020). In SCOS and SA, the predicted target genes of differentially expressed miRNAs were enriched in the Notch signaling pathway, and the downstream Notch effector Hes5 was significantly elevated in the seminal plasma of NOA patients, especially SA patients. Therefore, the Notch signaling pathway during spermatogenesis is related to the pathogenesis of NOA, and miR-34c-5p in seminal plasma may be a potential diagnostic marker of NOA (Zhang et al. 2021).

MiRNA-mediated regulation of the Wnt/β-catenin signaling pathway

The WNT protein family comprises a minimum of 19 secreted glycoproteins (Hayat et al. 2022). Upon binding of Wnt ligands to FZD-LRP5/6 receptors located on the cell surface, inhibition of β-catenin phosphorylation occurs, leading to its dissociation from the destruction complex. The destruction complex, comprising Axin, GSK-3β, CK1, and Adenomatous polyposis coli, orchestrates the proteasomal degradation of the β-catenin protein. Therefore, Wnt ligands suppress β-catenin degradation, leading to increased cytoplasmic accumulation (Hayat et al. 2022). Further, upon association with T cell factor/lymphoid enhancer factor proteins, β-catenin translocates to the nucleus, where it serves as a transcription activator capable of binding to DNA (Guo et al. 2021). The Wnt/β-catenin signaling pathway inhibits pro-apoptotic proteins while upregulating survivin, a member of the inhibitor of the apoptosis protein family, thereby exerting a protective effect against apoptosis (Sun et al. 2024).

Yang et al. found that miR-202-3p increased in the SCs of SCOS patients compared to OA patients with normal spermatogenesis. The upregulation of miR-202-3p led to apoptosis in SCs while concurrently suppressing cellular proliferation, whereas miR-202-3p depletion induced contrasting effects. Collectively, miR-202-3p directly targets lipoprotein receptor-related protein 6 (LRP6) and Cyclin D1 within the Wnt/β-catenin signaling pathway specifically within SCs, regulating proliferation and apoptosis of human SCs (Yang et al. 2019). It has been reported that miR-224 was expressed at a high level in mouse SSC and was involved in the regulation of self-renewal of SSCs. Meanwhile, miR-224 promotes SSCs differentiation by targeting DMRT1 and Wnt/β-catenin signaling (Cui et al. 2016).

MiRNA-mediated regulation of the mTOR signaling pathway

The mammalian target of rapamycin (mTOR) is a non-canonical serine/threonine protein kinase that integrates extracellular signals and phosphorylates downstream target protein ribosome p70S6 kinases, such as S6K1 and 4E-BP1, which play a key role in many transduction cascades of cell cycle progression and participate in the regulation of cell growth, proliferation, differentiation, apoptosis and other processes (Battaglioni et al. 2022; Shi et al. 2024a). Recent studies have found that the mTOR signaling pathway plays a key role in male fertility and is required for sperm proliferation and differentiation (Cao et al. 2020; Li et al. 2022). In addition, miRNAs can regulate cell proliferation and apoptosis through mTOR signaling (Akbarzadeh et al. 2021).

It has been reported that ultraviolet C (UVC) radiation can lead to decreased levels of reproductive hormones in the testis of rats, impaired spermatogenesis, and lower sperm quality, ultimately leading to decreased fertility in males. UVC radiation caused a significant downregulation of the expression level

of miR-20a-5p in the testis, and there was a significant correlation between the level of miR-20a and reproductive parameters exposed to UVC radiation (Alahwany et al. 2025). miR-20a is a member of the miR-17–92 family. The miR-17–92 cluster, encompassing six distinct mature miRNAs—namely miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a—exerts a significant influence on embryo development (Hurtado et al. 2024). Studies have found that the specific deletion of miR-17–92 within the testes of mature mice led to pronounced testicular atrophy, empty seminiferous tubules, and diminished spermatozoa production (Xie et al. 2016). The silencing of miR-17–92 has been correlated with hyperactivation of the mTOR signaling pathway, concomitant with the upregulation of the pro-apoptotic factors Stat3, Bim, Socs3, and c-Kit. These factors contribute in part to the observed phenotypic alterations in the testes of mutant specimens (Xie et al. 2016).

In summary, miRNAs may play an important role in male germ-cell apoptosis by regulating various signaling pathways such as PI3K/AKT, NOTCH, Wnt/β-catenin, and mTOR (Fig. 2). They fine-tune cell fate by targeting key apoptosis-related genes, providing potential targets for male reproductive health maintenance and disease treatment.

Competing endogenous RNAs: an important mediator of reproductive cell apoptosis

Competing endogenous RNAs (ceRNAs) refer to transcripts capable of mutual regulation at the post-transcriptional level by engaging in competition for common miRNAs. CeRNA networks establish a functional connection between protein-coding mRNAs and various classes of non-coding RNAs, including lncRNAs, circular RNAs, miRNAs, and pseudogenic RNAs (Zhong et al. 2018). Intracellularly, lncRNA and circRNA possess the ability to act as miRNA decoys through the presence of shared miRNA response elements, thereby exerting indirect

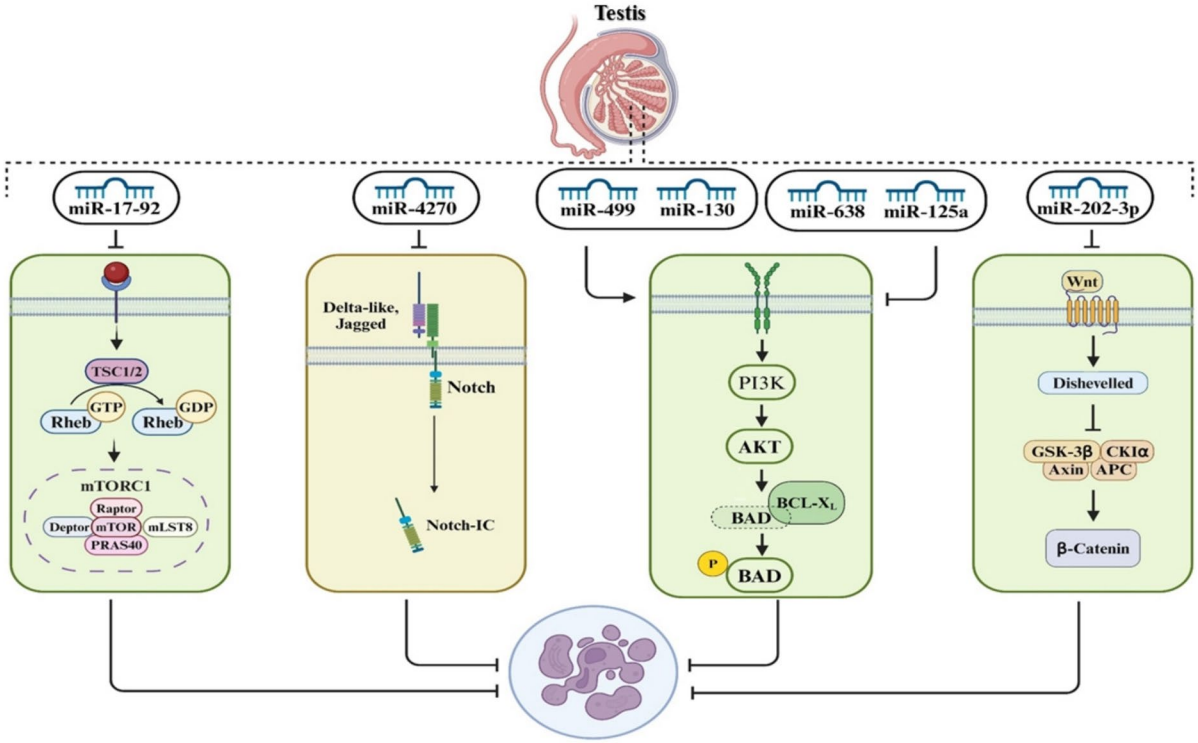

Fig. 2 A schematic representation of the interplay between microRNAs (miRNAs) and apoptosis-related signaling pathways, including PI3K/AKT, Wnt/β-catenin, NOTCH, and mTOR, in the regulation of apoptosis in male reproductive cells (the figure was created with BioRender.com)

control over the expression of downstream target genes (Ma et al. 2023). A growing body of evidence suggests that the ceRNA regulatory network plays a significant role in various biological processes related to infertility, including the apoptosis of reproductive cells. In the upcoming section, we will delve into the significance of ceRNAs and their role in regulating apoptosis within reproductive cells (Lv et al. 2024; Zhang et al. 2024b).

It has been reported that miR-122-5p exhibited direct targeting of CBL gene expression, thereby promoting the processes of proliferation and DNA synthesis within human SSCs, while concurrently suppressing the initiation of early apoptosis in this cellular population. However, lncRNA CASC7 engaged in competitive interaction with miR-122-5p, thereby mitigating the inhibitory effect on CBL expression (Zhou et al. 2020b). In GC-1 spermatogonia cells of mice, the heightened expression of miRNA-761 markedly repressed the expression levels of NANOS2 and HOTAIR, resulting in the inhibition of cellular proliferation and facilitation of apoptosis. HOTAIR can promote the proliferation and inhibit the apoptosis of apoptosis in murine spermatogonium GC-1 cells through the sequestration of miR-761, thereby regulating the expression of NANOS2 (Kong et al. 2022; Zhang et al. 2024a). As testicular ischemia–reperfusion injury (IRI) advances, there is an observed up-regulation of LncRNA MALAT1 in both testicular tissues and GC-1 cells. This up-regulation demonstrates a positive association with cell apoptosis and an inverse relationship with cell proliferation. MiR-214 operates as a specific target of MALAT1, while TRPV4 serves as a target of miR-214. Studies have found that lncRNA MALAT1 promotes apoptosis and inhibits cell proliferation during testis IRI by regulating miR-214 and TRPV4 pathways (Gong et al. 2020; Li et al. 2018b). The expression of lncRNA MIR22HG was found to be increased in the testicular tissues of mice exhibiting late-onset hypogonadism as well as in TM3 cells (a mouse Leydig cell line) subjected to H_2O_2. MIR22HG engages in direct interaction with miR-125a-5p, leading to the subsequent downregulation of N-Myc downstream-regulated gene 2 (NDRG2) expression through targeting of its mRNA 3′-UTR by miR-125a-5p, and exacerbated apoptosis and diminished testosterone synthesis (Liu et al. 2021b; Wang et al. 2022b; Yan and Wang 2025). Bian et al. found that high expression of CircBTBD7 increased cellular proliferation while concurrently suppressing apoptosis within immature porcine SCs. CircBTBD7 served as a competitive inhibitor by sequestering miR-24-3p, thereby promoting the expression of its downstream target, the mitogen-activated protein kinase 7 (MAPK7) gene, and inhibiting apoptosis of porcine SCs (Bian et al. 2021). LncRNAs can also provide miRNAs. In GC-1 spg cells, Dynamin 3 opposite strand serves as a supplier of pre-miRNA-214-5p, thereby promoting the upregulation of miR-214-5p expression and regulating the apoptosis and aging process of spermatogonium by decreasing the expression of E2F2. This suggests that it has the potential to be a novel therapeutic target for addressing male infertility through gene therapy (Hua et al. 2023).

In conclusion, ceRNAs can affect apoptosis-related signaling pathways maintain cell homeostasis through the adsorption of miRNAs, and play an important role in the apoptosis of male germ cells (Fig. 3). The ceRNAs study not only reveals new regulatory mechanisms but also provides potential targets for the diagnosis and treatment of male infertility.

Endogenous factors-induced reproductive toxicity: the emerging role of miRNA dysregulation in apoptosis induction

Diabetes-induced apoptosis: a new challenge in infertility

Diabetes mellitus (DM) is a chronic non-communicable disease. In males, DM can lead to infertility as it impacts numerous signaling pathways crucial for spermatogenesis (Barkabi-Zanjani et al. 2020). Several research investigations demonstrated that diabetes could trigger apoptotic alterations in the reproductive system through the modulation of non-coding RNAs, such as miRNAs. One study indicated that the miR-34a-specific inhibitor and the SIRT1 activator (SRT2104) notably mitigated oxidative stress, endoplasmic reticulum stress, and apoptotic cell demise in the testes. In this manner, the pivotal involvement of miR-34a/SIRT1 in DM-induced TACD underscores the potential therapeutic significance of miR-34a suppression and SIRT1 activation in the clinical intervention of DM-induced TACD and male infertility (Jiao et al. 2018). MiR-27b-3p was found to interact

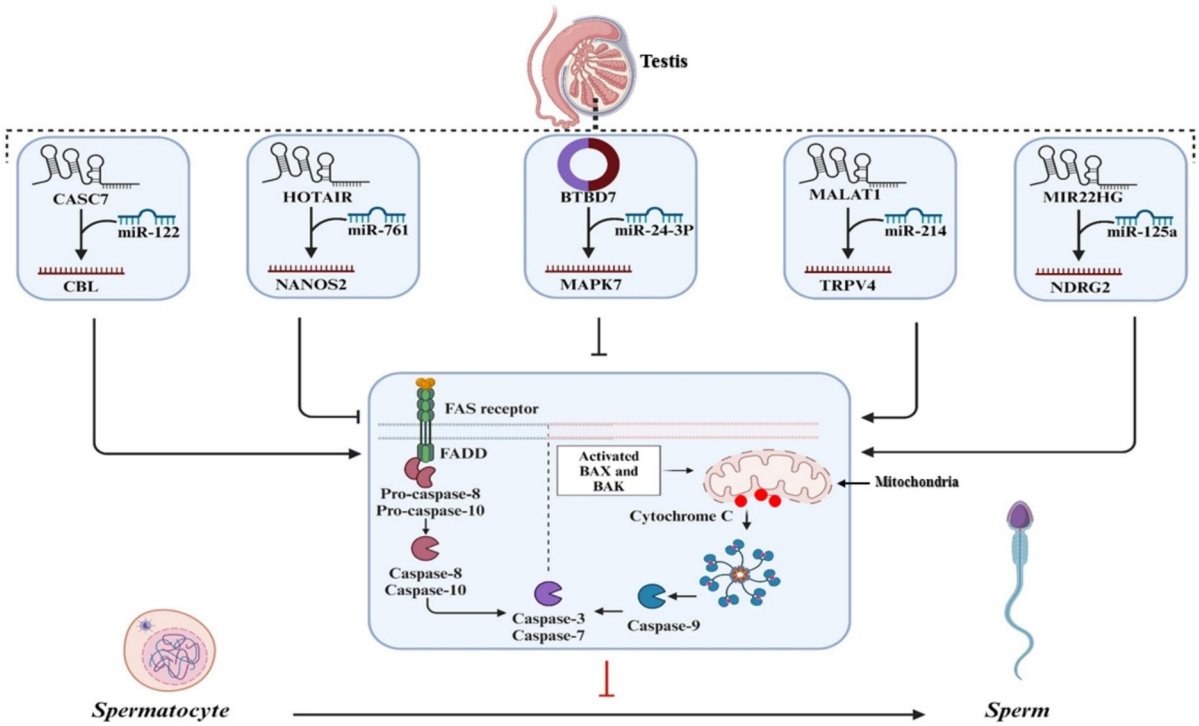

Fig. 3 Schematic representation illustrating the regulatory network of ceRNAs implicated in the modulation of male reproductive cell apoptosis (the figure was created with BioRender.com)

directly with Gfpt1 in GC-1 spermatogenic cells treated with HG, leading to the inhibition of hexosamine biosynthetic pathway (HBP) signaling. MiR-27b-3p has shown the potential to ameliorate HG-induced apoptosis and injury in spermatogenic cells by modulating Gfpt1/HBP signaling, thereby offering a novel therapeutic avenue for addressing diabetes mellitus-induced testicular damage (Zheng et al. 2022). Moreover, treatment with glucose may also impact the expression of miRNAs in LCs. MiR-504 and miR-935 mediate LC apoptosis via the classic survival pathway involving MEK5-ERK5-MEF2C, and attenuation of these miRNAs may potentially mitigate the apoptosis of LCs induced by high glucose levels (Hu et al. 2021).

Exploration of miRNAs in regulating apoptosis in testicular IRI

Testicular torsion, a critical urological ailment involving torsion of the spermatic cord, presents a significant risk of testicular damage, potentially resulting in male subfertility. Testicular torsion/detorsion is regarded as the principal pathophysiological event leading to IRI, resulting in increased apoptosis and dysfunction of testicular spermatogenesis (Al-Maghrebi et al. 2010; Arena et al. 2017). In the context of testicular IRI, miRNAs have been identified as crucial modulators of apoptotic processes in spermatogenic cells, orchestrating their influence through the regulation of pivotal target genes integral to the apoptotic signaling pathway. In this regard, Ning et al. explored the involvement of miR-29a in the IRI of the testes. As testicular IRI advanced, a discernible negative correlation was observed between the expression levels of miR-29a and TRPV4, with a notable downregulation of miR-29a evident in both animal specimens and GC-1 cells. Further, TRPV4 operates as a molecular target regulated by miR-29a. They observed that the suppression of miR-29a resulted in heightened TRPV4 expression and facilitated apoptotic processes in spermatogenic cells, while miR-29a overexpression led to decreased TRPV4 levels and mitigation of spermatogenic cell apoptosis induced by testicular IRI, both in vivo and in vitro. Thereby, miR-29a mitigates testicular IRI-induced apoptosis

by directly modulating TRPV4 expression (Ning et al. 2017). In conclusion, the complex interaction between testicular IRI and the regulatory functions of miRNAs highlights promising therapeutic strategies for attenuating testicular damage and preserving male fertility.

MiRNAs: A new player in heat stress-induced apoptosis in the testis

Heat stress (HS) arises from temperatures surpassing a physiological threshold, leading to an overload of compensatory mechanisms. HS, by triggering apoptosis, could potentially result in the depletion of testicular germ cells, consequently influencing male fertility (Cai et al., 2021). It has been reported that HS is associated with a reduction in miR-128-3p expression, elevated p-MAPK14 expression, and enhanced apoptosis. In the GC2-spd cell line cultured in vitro, inhibition of miR-128-3p leads to the upregulation of p-MAPK14 expression, attenuates cellular proliferation, and enhances apoptosis, aligning with findings observed in heat treatment alone. Furthermore, following HS, the transfection of GC2 cells with miR-128-3p mimics led to a reduction in p-MAPK14 expression, mitigation of decreased cell proliferation, and attenuation of apoptosis levels. In this manner, testicular hyperthermia leads to apoptosis of spermatogenic cells and detrimentally affects spermatogenesis by downregulating miR-128-3p and facilitating MAPK14 phosphorylation (Zou et al. 2021). Sperm production is highly temperature dependent. The study found that HS caused the mice to lose testicular weight and sperm density. HS can lead to testicular atrophy and spermatogenesis by affecting the meiosis process and cell cycle, among which miR-143-3p may be a key regulatory factor affecting spermatogenesis (Gan et al. 2023). In addition, under HS, miR-199a-3p can regulate the proliferation of mouse sperm stem cells by targeting ID4 (Zhuo et al. 2023).

MiRNAs: a new player in hypoxia-induced apoptosis in the testis

Hypoxia is defined as a temporary or sustained state marked by diminished arterial oxygen partial pressure leading to tissue oxygen deprivation, accompanied by a reduction in arterial oxygen partial pressure and oxygen content. Hypoxia has been demonstrated to detrimentally impact male fertility in both animals and humans, potentially resulting in a diminished sperm count, decreased sperm motility, and aberrant sperm morphology in ejaculate (Ata-Abadi et al. 2020; Wang et al. 2021). The potential pathways implicated in hypoxia-induced male reproductive toxicity primarily encompass heightened ROS mediated by oxidative stress and germ cell apoptosis mediated by HIF-1α. Recent experiments demonstrated that suppressing the HIF-1α gene in the testes of VC rats reduces apoptosis in spermatogenic cells and significantly enhances testicular spermatogenesis (Zhao et al. 2019). It has been reported that hypoxia prompted apoptosis in GC-2 cells and upregulated the expression of HIF-1α and pro-apoptotic proteins while concurrently reducing the levels of anti-apoptotic proteins (Li et al. 2021b). The master hypoxemia, miR-210, is considered the primary driver of the cellular response to hypoxic stress (Chan et al. 2012). Another study found that under hypoxic conditions, miR-210 upregulation triggered the apoptotic pathway in mouse GC-2 cells via KLF7 targeting (Lv et al. 2019) It indicated that hypoxia can regulate the expression of specific miRNA-and play an important role in hypoxia-induced apoptosis of testicular cells.

Endogenous factors (such as diabetes, IRI, heat stress, and hypoxia) affect the apoptosis of male germ cells by regulating the expression pattern of miRNAs, which is an important cause of male infertility (Fig. 4). Regulating the expression or function of miRNAs may provide novel therapeutic strategies for male infertility caused by diabetes, IRI, heat stress, and hypoxia.

External factor-induced reproductive toxicity: the emerging role of miRNA dysregulation in apoptosis induction

Early-life exposure and later-life health outcomes

Numerous epidemiological investigations have demonstrated that the early stages of human development significantly influence the susceptibility to non-communicable diseases in later stages of life. Endocrine-disrupting chemicals (EDCs) act as regulators of the endocrine and metabolic systems, disrupting pivotal developmental processes in mammals (Ghosh et al. 2022; Hameed et al. 2020). Siddeek et al. developed

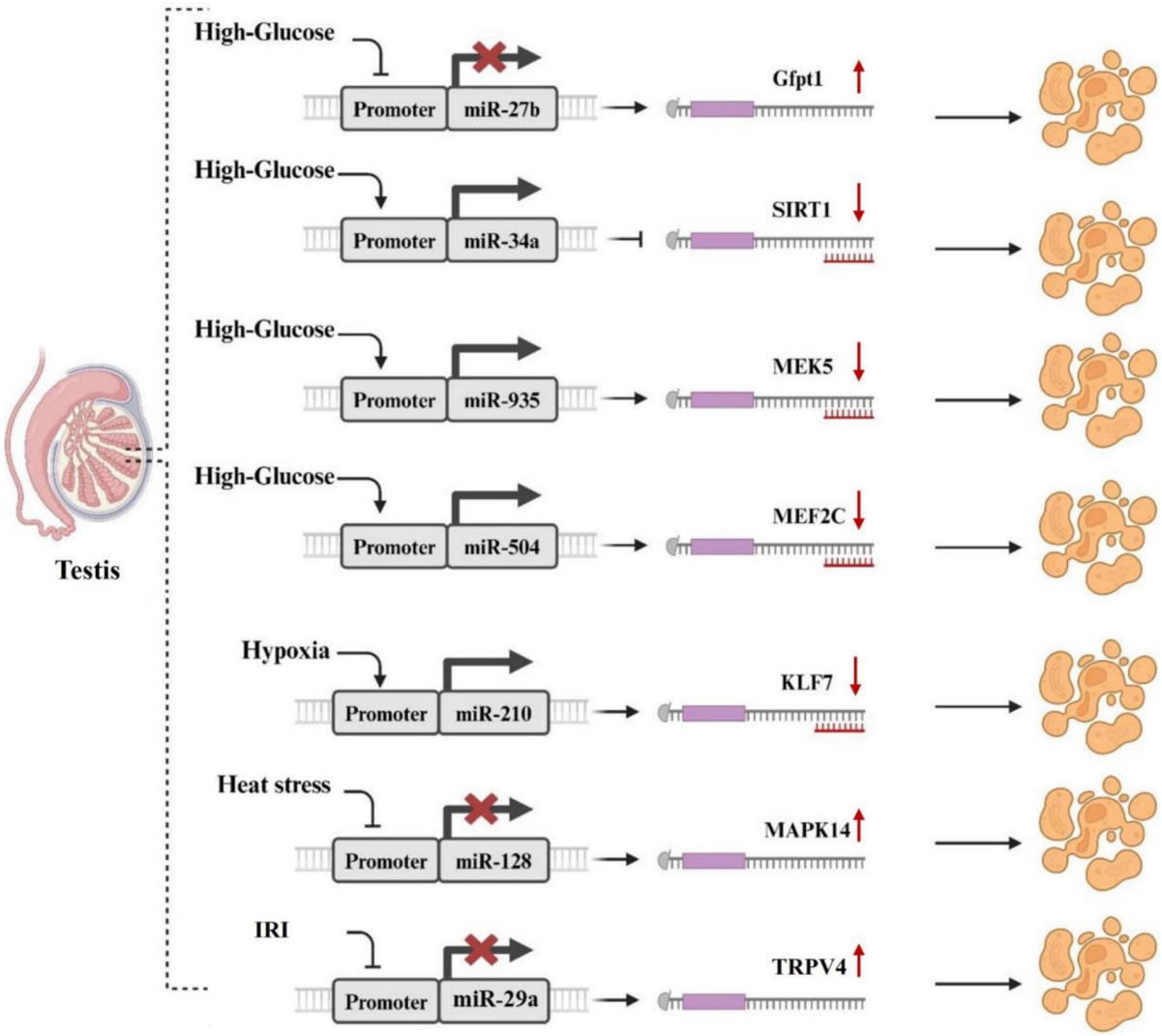

Fig. 4 Schematic representation illustrating the interplay between diabetes, hypoxia, IRI, and HS in male reproductive cells, mediated by miRNAs, leading to apoptotic pathway activation (the figure was created with BioRender.com)

an animal model with induced adult germ cell death disease through neonatal exposure to oestradiol benzoate (EB), they uncovered a reciprocal negative feedback mechanism characterized by a reduction in EZH2 protein levels concomitant with augmented miR-101 expression. Their subsequent experimental investigations revealed that EZH2 silencing triggered apoptosis in germ cells by elevating apoptotic factors (BAD and BIM) and altering DNA repair through the deregulation of topoisomerase 2B. As well, elevated levels of miR-101 detected in animal blood suggest its potential utility as a circulating marker for germ cell death. In this manner, dysregulation of the miR-101–EZH2 pathway may signify a novel pathophysiological epigenetic foundation for adult germ cell disorders stemming from environmental and developmental factors (Siddeek et al. 2016). Furthermore, neonatal exposure to EB elicited apoptosis in adult germ cells alongside a dose-dependent elevation in the expression of miR-29a, miR-29b, and miR-29c. DNA methyltransferase (DNMT) is an enzyme responsible for maintaining and propagating DNA methylation patterns in proliferating cells (Laranjeira et al. 2023). Neonatal exposure to EB induced

long-term alteration of miR-29 in the testis of adult rats, resulting in reduced levels of DNMT and Mcl-1 proteins and underpinning the apoptotic phenotype of germ cells observed in adulthood (Meunier et al. 2012).

Adult exposure and reproductive toxicity

Between 1973 and 2018, the average sperm count of men worldwide fell by 62% and sperm concentration by 52%. This trend is universal across the globe (Levine et al. 2023). Bad lifestyles, drugs, and environmental poisons, such as silica nanoparticles (SiNPs), cyclosporine A (CsA), Cigarette smoke (CS), nonylphenol (NP), and ochratoxin A (OTA), have been implicated in reproductive toxicity through the induction of apoptosis, often involving miRNA dysregulation. SiNPs, for example, modulate miRNAs like miR-2861 and miR-450b-3p to activate apoptosis pathways in spermatogenic cells, including the Fas/FasL/RIPK1 and caspase cascades (Ren et al. 2019; Zhou et al. 2021). Similarly, CsA upregulates miR-34a, which triggers testicular damage via the miR-34a/sirtuin-1 axis (Ghazipour et al. 2020). Chronic CS exposure leads to testicular apoptosis through miR-138-5p modulation, suppressing key apoptotic regulators such as Caspase-3, Bak, and p53 (He et al. 2021b). NP exposure reduces miR-361-3p levels, promoting apoptosis in spermatogonia through the Killin/Tp73/Puma pathway (Tang et al. 2017). OTA induces apoptosis in GC-2 cells via miR-122 upregulation, activating caspase-3 and reducing Bcl-w expression (Chen et al. 2022, 2015). The decline in male fertility requires the attention of all sectors of society, especially in terms of public health and environmental protection. For individuals, maintaining a healthy lifestyle is one of the most effective ways to prevent and improve male reproductive decline. At the same time, it is urgent to develop targeted treatments for the main causes of reduced male fertility.

Male germ cells are affected by various environmental factors, and elucidation of their regulatory mechanisms is of great significance for the prevention and treatment of male reproductive system diseases related to apoptosis. The altered expression of miRNAs is associated with multiple adverse environmental exposures and may be a potential diagnostic marker and therapeutic target for male infertility (Fig. 5).

Research progress on alleviating reproductive cell apoptosis: from traditional to molecular therapy

Germ cell apoptosis is one of the important causes of male infertility, and its mechanism involves a variety of signaling pathways and molecular regulatory networks. In recent years, the research of alleviating germ cell apoptosis has gradually developed from traditional therapy to molecular therapy and has made remarkable progress. Although the current studies are limited to the animal level, we believe that future miRNAs targeted therapies for male infertility will make progress.

Alleviating reproductive cell apoptosis with traditional therapy

Traditional therapy mainly alleviates germ cell apoptosis through antioxidants, hormone regulation, and lifestyle changes. Lycopene, a hydrocarbon carotenoid, has emerged as a promising candidate for the prevention of human prostate cancer and cardiovascular disease (Mirahmadi et al. 2020; Riccioni et al. 2008). The identification of lycopene as a powerful antiapoptotic agent and its preventive effects against oxidative stress have prompted researchers to explore its potential to safeguard sperm cells from apoptosis, which can result in infertility. In this regard, it was demonstrated that lycopene has the potential to reduce hypoxia-induced apoptosis in GC-2 cells and can increase the expression of miR-23a/b in both in vivo and in vitro hypoxia models. Mimics of miR-23a and miR-23b may reduce hypoxia-induced apoptosis in GC-2 cells, likely through targeting of prokineticin 2 (PROK2) mRNA. In this manner, lycopene mitigates spermatocyte apoptosis induced by hypoxia via the miR-23a/b–PROK2 signaling pathway (Wang et al. 2024). Vitamin D3 has a significant effect on improving sperm motility and morphology. Mohamed DI et al. found that vitamin D3 can improve the IRI induced by testicular torsion in rats by up-regulating the expression of miR-145 and targeting the expression of apoptosis marker ADAM17 (Mohamed et al. 2021). Natural plants are widely used in traditional medicine. Kaempferia parviflora is a medicinal plant with antioxidant and anti-inflammatory activity, capable of treating male sexual dysfunction. It is reported that testosterone, sperm count, motor ability, and sperm viability of male rats treated with

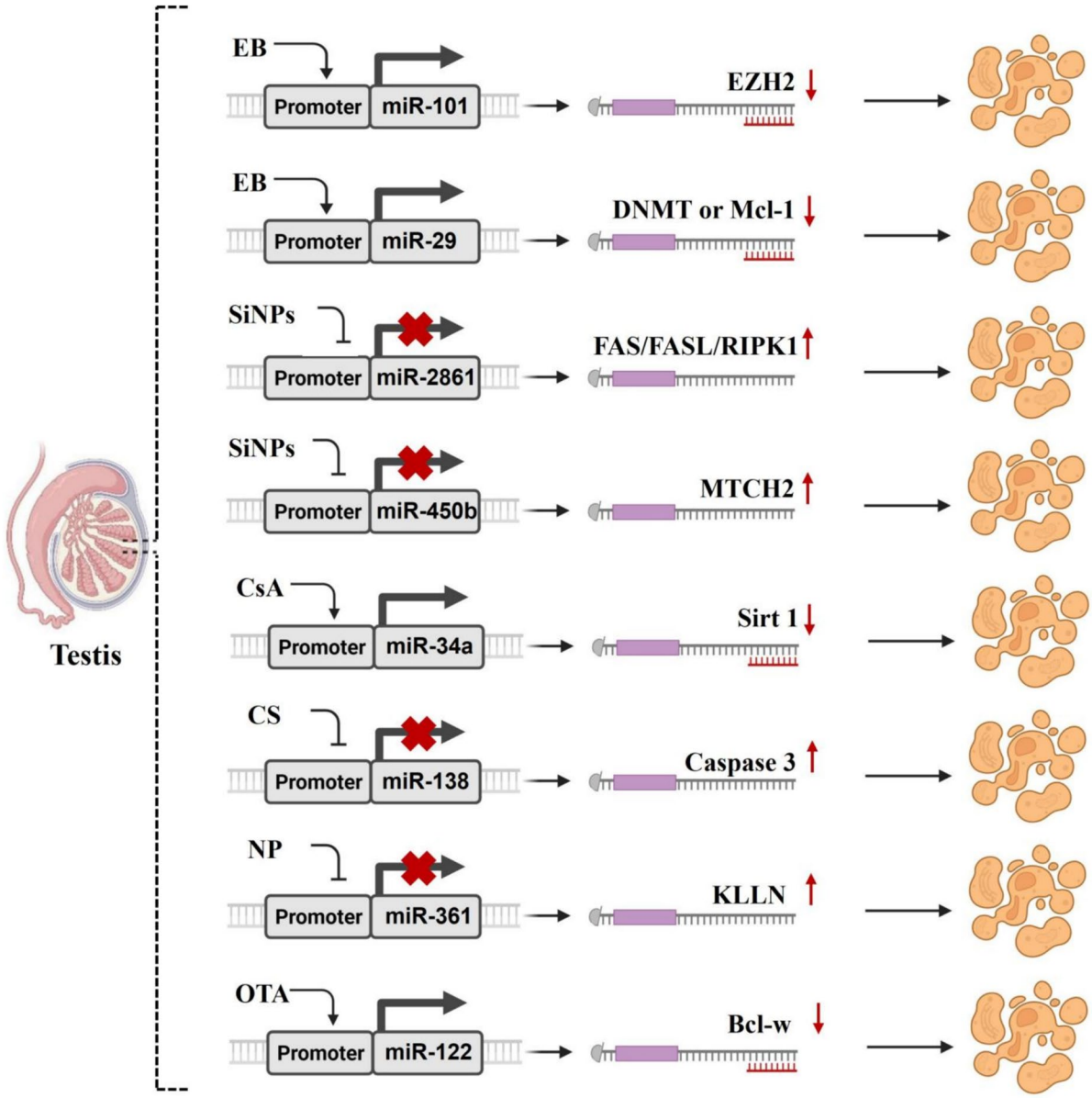

Fig. 5 A schematic representation of the exposure to adverse environmental factors, such as oestradiol benzoate (EB), silica nanoparticles (SiNPs), cyclosporine A (CsA), cigarette smoke (CS), nonylphenol (NP), and ochratoxin A (OTA), which mediates activation of the male reproductive cell apoptosis pathway through miRNAs targeting (the figure was created with BioRender.com)

Kaempferia parviflora were significantly increased, which may be related to the increased expression of miR-34 and the decreased expression of miR-328 and miR-19b (Al-Rawaf et al. 2021). The development of normal sperm depends on the regulation of gonadotropins (such as FSH, and LH). Gonadotropin can reduce germ cell apoptosis by regulating testosterone levels and SC function. It was found that miRNA subpopulations expressed by rat SCs, including miR-23b, −30c, −30d, and −690, were stimulated during acute hormone inhibition and targeted signaling pathways (MAPK, ErbB, and TGF signaling pathways) and intercellular adhesion processes (Nicholls et al. 2011). In addition, a study demonstrated the presence

of mirNA-targeting components of the ERK pathway that are affected by FSH action in rats, including miR-30c and miR-30 (Crépieux et al. 2001).

Exercise and physical activity have been acknowledged for their role in promoting health and postponing the onset of various pathological conditions. As well, exercise is postulated to exert beneficial effects on testicular function through mechanisms including the upregulation of steroidogenic enzymes and antioxidant defenses, alongside the downregulation of inflammatory pathways (Adelowo et al. 2024; Gomes et al. 2015; Moreira et al. 2025). SIRT1, an NAD^+-dependent deacetylase, inhibits p53 activity by deacetylating the p53 protein at the post-transcriptional level. A recent experiment revealed an upregulation of testicular miR-34a and Ac-p53 expression, accompanied by a downregulation of SIRT1 expression, in the testes of diabetic rats (Gaderpour et al. 2021). It was further found that voluntary exercise enhanced spermatogenesis and reduced testicular apoptosis in rats by reducing oxidative stress and modulation of the miR-34a/SIRT1/p53 pathway (Gaderpour et al. 2021). Another study on a high-fat diet also found that voluntary exercise reduced testicular oxidative stress and apoptosis induced by a high-fat diet by altering the miR-34a/sirt1/p53 pathway, and improved spermatogenesis in rats (Heydari et al. 2021). In addition, eating a healthy diet and reducing the damage to the testicles from hot environments (e.g. saunas, hot baths) may also help improve male fertility.

Alleviating reproductive cell apoptosis with molecular therapy

MiRNAs are promising candidates for the future of personalized medicine. By injecting specific dysregulation states caused by miRNAs when down-regulated (using miRNA mimics), or delivering inhibitors when up-regulated, apoptosis-related signaling pathways and molecular regulatory networks can be targeted to precisely mitigate germ cell apoptosis. At present, anti-miRNA therapy and miRNA mimics have been mainly tested in various cancers and some other diseases but not for male infertility (Chakraborty et al. 2017). Exosomes, nanosized (30–90 nm) extracellular vesicles, arise through the multivesicular body sorting pathway. A diverse range of cell types can produce these vesicles. Exosomes function as intercellular messengers, delivering their encapsulated cargo to recipient cells and thereby influencing their behavior. Research suggests that exosomes hold promise as versatile therapeutic agents with potential applications across a broad spectrum of diseases, including reproductive disorders. Gao and colleagues observed that exosomes originating from mouse SCs exerted a suppressive effect on apoptosis in primary spermatogonia. They found that the level of miR-10b in exosomal miRNAs was significantly increased, and miR-10b could reduce the apoptosis of spermatogonium in both primary spermatogonia and the C18-4 cell line by downregulating its target KLF4 (Gao et al. 2023). Therefore, exosomes, shown to carry miRNAs that suppress apoptosis in spermatogonia, offer a promising avenue for therapeutic intervention in reproductive disorders.

Nanoparticle delivery systems can be used to deliver miRNAs, siRNAs, or small molecule drugs to improve targeting and therapeutic effectiveness. High testicular temperature harms male fertility, and oxidative stress is one of the main reasons. The study found that high testicular temperature in rats led to decreased Bcl-2 gene expression and increased expression of Bax, miRNA-21, and CIRCRNA0001518 while containing curcumin, Vitamin D and E, and iron (III) oxide nanoparticles and manganese oxide nanoparticles can be improved due to high temperature of the scrotum rats decreased fertility (Khosravi et al. 2020). Sertoli cell-derived small extracellular vesicles (SC-sEV) can enter the testes through the blood and cross the blood-testis barrier to enter germ cells. Chen et al. loaded a miR-24-3p inhibitor into SC-sEV to create a nanomedical SC-sEV@miR-24-3p inhibitor. SC-sEV@miR-24-3p inhibitor delivers miR-24-3p inhibitor to germ cells, effectively cancels the inhibitory effect of miR-24-3p on GSK3β expression, improves energy metabolism and alleviates oxidative stress in rat germ cells (Rastogi et al. 2023).

In conclusion, traditional therapies improve germ cell survival through antioxidants, hormone regulation, and lifestyle interventions, but the efficacy is limited. Molecular therapy can precisely reduce germ cell apoptosis and has broad application prospects. Future studies should further explore the safety and effectiveness of molecular therapy, develop more accurate treatment strategies, and provide personalized treatment plans for male infertility patients. At the same time, the combination of traditional therapy and molecular therapy may be an important direction to alleviate germ cell apoptosis (Fig. 6).

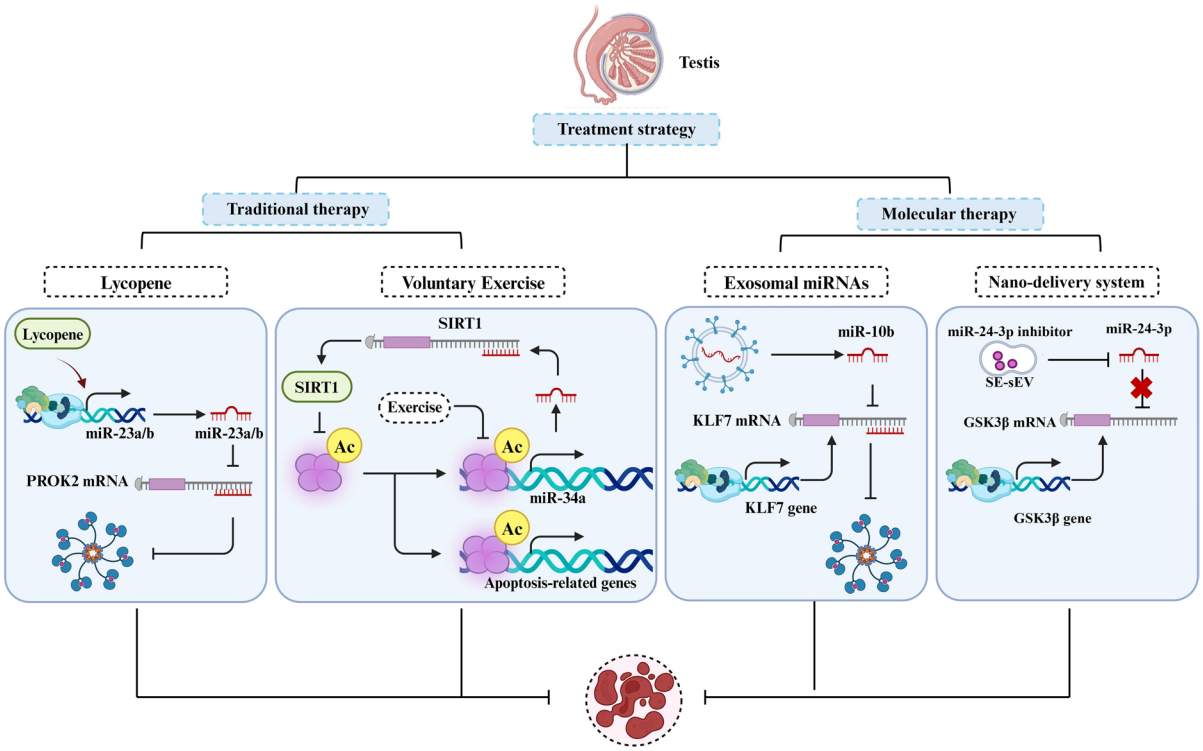

Fig. 6 Schematic representation illustrating the therapeutic potential of modulating miRNAs in alleviating apoptosis of reproductive cells through traditional and molecular approaches (the figure was created with BioRender.com)

Limitations and directions for future research

Although the important role of miRNAs in male germ cell apoptosis and infertility has been extensively studied, there are still some limitations in the current research. First, most studies are based on animal models or in vitro experiments, and the specific regulatory mechanisms in humans still need to be further verified (Tables 1 and 2). Second, the expression of miRNAs is tissue-specific and individual, and the results of different studies may be inconsistent. Second, the expression of miRNAs is tissue-specific and individual, and the results of different studies may be inconsistent. In addition, the regulatory networks of miRNAs are complex, and their target genes and signaling pathways have not been fully elucidated, which poses challenges for the development of precision therapeutic strategies. Finally, mirNAS-based therapies are still in the experimental stage, and questions about their safety, efficacy, and delivery systems need to be addressed. Future studies are needed to combine multi-omics techniques and clinical data to

Table 1 Major non-coding RNAs in human reproductive cell apoptosis (clinical trial)

Non-coding RNAs	Target	Expression levels	Species	Cell type	Influence on apoptosis	Ref
MiR-210-3p	-	Increase	Human	Germ cells (GC-2)	Promoting	(Xu et al. 2020b)
MiR-34b-5p	ITPR1	Decrease	Human	Testicular tissue	Inhibiting	(Maleki et al. 2023)
MiR-4270	GADD45A	Increase	Human	Sertoli cells	Promoting	(Wang et al. 2020b)
MiR-202-3p	LRP6 and Cyclin D1	Increase	Human	Sertoli cells	Promoting	(Yang et al. 2019)

Table 2 Major non-coding RNAs in reproductive cell apoptosis (animal/cell experiments)

Non-coding RNAs	Target	Expression levels	Species	Cell type	Influence on apoptosis	Ref
MiR-210	KLF7	Increase	Mouse	Spermatocyte (GC-2 cells)	Promoting	(Lv et al. 2019)
MiR-322	Ddx3x	Decrease	Human	Germ cells (GC-2)	Inhibiting	(Che et al. 2019)
MiR-130a	Androgen receptor	Increase	Mouse	Sertoli cell	Promoting	(Li et al. 2018a)
MiR-100-3p	SGK3	-	Human	Sertoli cell	Inhibiting	(Liu et al. 2021a)
MiR-362	RMI1	-	Porcine	Sertoli cells	Promoting	(Ran et al. 2020)
MiR-26a	PAK2	-	Porcine	Sertoli cells	Promoting	(Ran et al. 2018)
MiR-196a	RCC2 and ABCB9	-	Porcine	Sertoli cells	Inhibiting	(Zhang et al. 2019)
MiR-34c	ATF1	-	Mouse	Germ cells (GC-2)	Promoting	(Liang et al. 2012)
MiR-34a	SIRT1	-	Mouse	Testicular tissue	Promoting	(Jiao et al. 2018)
MiR-31-5p	JAZF1 and Cyclin A2	-	Human	Spermatogonial stem cells	Promoting	(Fu et al. 2019)
MiR-1908-3p	KLF2	-	Human	Spermatogonial stem cells	Inhibiting	(Chen et al. 2020)
MiR-663a	NFIX	-	Human	Spermatogonial stem cells	Inhibiting	(Zhou et al. 2018)
MiR-22-5p	EZH2	Increase	Human	Spermatogonial stem cells	Promoting	(Lv et al. 2022)
MiR-21	-	-	Mouse	Spermatogonial stem cells	Inhibiting	(Niu et al. 2011)
MiR-188-3p	MLH1	Decrease	Mouse	Spermatogenic cells	Inhibiting	(Song et al. 2017)
MiR-10b	KLF4	-	Mouse	Spermatogonial stem cells	Inhibiting	(Li et al. 2017)
MiR-30a-5p	-	-	Mouse	Spermatogonial stem cells	Inhibiting	(Khanlari et al. 2021)
MiR-638	SPAG1	-	Porcine	Sertoli cells	Promoting	(Hu et al. 2017)
MiR-126	PIK3R2	-	Porcine	Sertoli cells	Inhibiting	(Tang et al. 2021)
MiR-499	PTEN	-	Porcine	Sertoli cells	Inhibiting	(Gao et al. 2020a)
MiR-125a-5p	RAB3D	-	Mouse	Sertoli cells	Promoting	(Teng et al. 2021)
MiR-17–92	Bim and Stat3	Decrease	Mouse	Spermatogonial stem cells	Inhibiting	(Xie et al. 2016)
MiR-27b-3p	Gfpt1	Decrease	Mouse	Spermatogenic cells (GC-1 spg cells)	Inhibiting	(Zheng et al. 2022)
MiR-29a	TRPV4	Decrease	Mouse	Spermatogenic cells (GC-1 spg cells)	Inhibiting	(Ning et al. 2017)
MiR-128-3p	MAPK14	Decrease	Mouse	Germ cells (GC-2)	Inhibiting	(Zou et al. 2021)
MiR-2861	Fas/FasL/RIPK1	Decrease	Mouse	Spermatocyte cells (GC-2spd)	Inhibiting	(Ren et al. 2019)
MiR-450b-3p	MTCH2	Decrease	Mouse	Spermatocyte cells (GC-2spd cells)	Inhibiting	(Zhou et al. 2021)
	Layilin	Decrease	Mouse	Spermatocyte cells (GC-2spd cells)	Inhibiting	(Zhou et al. 2023)
MiR-5622-3p	ZCWPW1	Increase	Mouse	Spermatogenic cells	Promoting	(Zhao et al. 2023)
MiR-138-5p	Caspase-3	Decrease	Mouse	Leydig and Sertoli cells	Inhibiting	(He et al. 2021b)

Table 2 (continued)

Non-coding RNAs	Target	Expression levels	Species	Cell type	Influence on apoptosis	Ref
MiR-361-3p	Klln	Decrease	Mouse	Spermatocyte (GC-1 cells)	Inhibiting	(Tang et al. 2017)
MiR-23a/b	PROK2	-	Mouse	Spermatocyte (GC-2 cells)	Inhibiting	(Wang et al. 2024)
LncRNA CASC7	By sponging miR-122, suppressed the inhibition of CBL	Decrease	Human	Spermatogonial stem cells	Inhibiting	(Zhou et al. 2020b)
LncRNA HOTAIR	By sponging miR-761, suppressed the inhibition of NANOS2	-	Mouse	Germ cells (GC-2)	Inhibiting	(Kong et al. 2022)
LncRNA MALAT1	By sponging miR-214, suppressed the inhibition of TRPV4	Increase	Mouse	Germ cells (GC-2)	Promoting	(Li et al. 2018b)
LncRNA MIR22HG	By sponging miR-125a-5p, suppressed the inhibition of NDRG2	Increase	Mouse	Leydig cells	Promoting	(Liu et al. 2021b)
CircBTBD7	By sponging miR-24-3p, suppressed the inhibition of MAPK7	-	Porcine	Sertoli cell	Inhibiting	(Bian et al. 2021)

fully uncover the role of miRNAs in male infertility and advance its translation to clinical applications.

Conclusion

Male infertility is a complex condition with a variety of contributing factors involved. Emerging evidence suggests that germ cell apoptosis, encompassing SSCs, SCs, and LCs, is an important factor in male infertility. miRNAs, as post-transcriptional regulators, are crucial in modulating apoptosis. In the current work, we reviewed the bifunctional nature of miRNAs in apoptosis and the effects of their dysregulation on male germ cells. For example, several miRNAs, including miR-210-3p, miR-322, miR-130a, miR-26, miR-31p, and miR-22-5p, promote apoptosis. Other miRNAs, such as miR-100-3p, miR-362, miR-196a, miR-1908, miR-663, and miR-21 exhibit anti-apoptotic functions. Moreover, several signaling pathways, such as PI3K/AKT, Wnt/β-catenin, Notch, and mTOR, known to regulate apoptosis, are amenable to control by miRNAs. Notably, in this review, we found that various internal factors (such as diabetes, hypoxia, heat stress, and testicular IRI) and external factors (EDCs, SiNPs, CsA, cigarette smoke, nanoparticles, and OTAs) induce male infertility through miRNAs-mediated apoptosis. Finally, we highlighted those traditional treatments such as antioxidants, hormone regulation, and lifestyle changes, as well as novel therapeutic medicine such as exosome and nanoparticle delivery systems, provide new directions for improving male reproductive health by modulating miRNAs and protecting reproductive cells from apoptosis. Future studies should further reveal the specific regulatory network of miRNAs in male infertility and explore its clinical application potential to lay a foundation for the precise treatment of male infertility.

Abbreviations 3′UTR: 3′ Untranslated region; APO: Apoptosis antigen; AR: Androgen receptor; ATF1: Activating transcription factor 1; Bcl-2: B-cell lymphoma 2; Ca^{2+}: Calcium; CCNE1: Cell cycle regulators like cyclin-E1; CCND1: Cyclin-D1; CDK4: C-MYC and cyclin-dependent kinase 4; ceRNAs: Competing endogenous RNAs; circRNA: Circular RNA; CS: Cigarette smoking; CsA: Cyclosporine A; DNMT: DNA methyltransferase; DM: Diabetes mellitus; E2F2: E2 factor family of transcription factor 2; EB: Oestradiol benzoate; EDCs: Endocrine-disrupting chemicals; EGF: Epidermal growth factor; EZH2: Enhancer of zeste homolog 2; HBP: Hexosamine biosynthetic pathway; HES1: Hes family bHLH transcription factor 1; HS: Heat stress; ITPR1: Inositol 1,4,5-Trisphosphate Receptor Type 1; IRI: Ischemia-reperfusion injury; KLF2: Kruppel-like factor 2; lncRNAs: Long non-coding RNAs; LCs: Leydig cells; LRP6: Lipoprotein receptor-related protein 6; MAPK7: Mitogen-activated protein kinase 7; mTOR: Mammalian target of rapamycin; mRNA: Messenger RNA; Mcl-1: Myeloid cell leukaemia sequence 1; miRNAs: MicroRNAs; NDRG2: N-Myc downstream-regulated gene 2; NICD: Notch intracellular domain; NOA: Non-obstructive azoospermia; OA: Obstructive azoospermia; OTA: Ochratoxin A; PAK1: P21-activated kinase 1; PI3Ks: Phosphatidylinositol-3 kinases; PTEN: Phosphatase and tensin homolog; PROK2: Prokineticin 2; RAB3D: Ras-related protein Rab-3D; RMI1: RecQ-mediated genome instability protein 1; RNAs: Ribonucleic acids; SCOS: Sertoli cell-only syndrome; SCs: Sertoli cells; SC-sEV: Small extracellular vesicles; SGK3: Serum/glucocorticoid-regulated kinase family member 3; SiNPs: Silica nanoparticles; SMAD7: SMAD family member 7; SSCs: Spermatogonial stem cells; TNF: Tumor necrosis factor; UVC: Ultraviolet C

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Declarations

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