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REVIEW

MicroRNAs as diagnostic biomarkers in diabetes male infertility: a systematic review

Zeinab Latifi[1] · Saba Nikanfar[2] · Rasa Khodavirdilou[3] · Sohrab Minaei Beirami[4,5] · Lida Khodavirdilou[3] · Amir Fattahi[6] · Farnaz Oghbaei[7]

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Abstract

This study conducts an in-depth review of the correlation between testis tissue changes and circulating microRNAs (miRNA) in diabetes-induced male reproductive complications, drawing upon both animal and clinical studies. The original articles published in English that specifically investigate miRNAs linked to male infertility in humans or animals with either type I or II diabetes mellitus were included. The relevant articles were gathered from the PubMed, Google Scholar, Cochrane Library, and ScienceDirect databases. The quality of study was assessed utilizing the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Prevalence Studies. We collected an overall number of 1989 citations relating to our research subject. Following the elimination of articles based on the criteria, a total of 20 papers were included in the study. Aberrant expression profiles of 25 miRNAs were identified in diabetes associated with male reproductive issues, with 15 miRNAs exhibiting increased expression and 10 miRNAs showing decreased expression. Among the chosen publications, eighteen were identified as low-risk and two were classed as moderate quality. The dysregulated miRNAs were linked to testicular injury, disrupted steroid production, decreased sperm development and quality, and erectile dysfunction. The results demonstrate that the miRNA–mRNA network is linked to the pathological progression of diabetic testicular damage or erectile dysfunction. From a therapeutic perspective, the identification of circulating miRNAs could be beneficial in the timely identification and prevention of diabetes problems, such as diabetes-induced male infertility. Among all signaling pathways influenced by modified miRNAs, the Bax-caspase-3, MAPK, PI3K-Akt, and eNOS-cGMP-PKC were the main deregulated pathways.

Summary sentence

The expression of potential miRNA as diagnostic biomarkers may be evaluated in diabetes complications and might constitute a monitoring factor in the diabetes-induced male reproductive complications.

Keywords MicroRNAs · Diabetes · Erectile dysfunction · Spermatogenesis · Steroidogenesis · Testes

Abbreviations

HPG	hypothalamus-pituitary-gonadal
ncRNAs	non-coding RNAs
miRNAs	microRNAs
PRISMA	preferred reporting items for systematic reviews and meta-analysis
JBI	joanna briggs institute
ICP	intracavernous pressure
MAP	mean arterial pressure,,
TBARS	thiobarbituric acid reactive substances
α-SMA	smooth muscle actin
Grp78	glucose-regulated 78
Chop	C/EBP homologous protein
Gfpt1	glutamine-fructose-6-phosphate transaminase 1
HBP	hexosamine biosynthesis pathway

Zeinab Latifi and Saba Nikanfar contributed equally to this work and should be considered co-first authors.

Extended author information available on the last page of the article

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SRT2104	SIRT1-selective activator
eNOS	endothelial nitric oxide synthase
nNOS	neuronal nitric oxide synthase

Introduction

Diabetes poses a long-lasting and widespread health problem on a global scale. In 2021, the prevalence of diabetes among the global adult population was 10.5%, which corresponds to over 537 million individuals [1]. Diabetes mellitus has adverse effects on the male reproductive system by disrupting the hypothalamus-pituitary-gonadal (HPG) axis, impairing androgen secretion, interfering with spermatogenesis, and disrupting sexual behaviors, finally resulting in infertility. Infertility is a possible enduring consequence of diabetes [2]. Infertility is defined by the inability to achieve a pregnancy after 12 months or more of regular and unprotected sexual intercourse. Global infertility prevalence estimated in 2022 show that approximately one in six people have experienced infertility [3]. Infertility or subfertility has been reported in approximately 90% of patients with diabetes [4]. Diabetes can contribute to reproductive issues by modifying the levels of DNA and histone acylation, histone methylation, and non-coding RNAs (ncRNAs), specifically microRNAs (miRNAs) [5]. Recent findings indicate that miRNAs have the potential to serve as innovative biomarkers for diagnosing and treating complications of diabetes [6, 7].

MiRNAs are short non-coding RNA molecules consist of 21–24 nucleotides. They have important functions in controlling many cell processes such as cell growth, differentiation, and programmed cell death [8]. More than 1000 miRNAs have been discovered in humans, which selectively regulate almost 60% of genes found in mammals [9]. A single miRNA has the ability to target several transcripts, whereas multiple miRNAs can regulate a single gene. This suggests that even a little alteration in miRNA can play a role in the development of disorders such as diabetes [10]. These biomarkers, such as heart-specific miR-499, liver-specific miR-33, miR-122 [11–13], and testis-specific miRc-19, are specific to certain tissues and can be detected without the need for invasive procedures. These biomarkers are found in body fluids like plasma, where they are released in extracellular vesicles. This suggests that the presence of miRNA in the bloodstream may signal the level of activity of cells or the extent of tissue damage in illness conditions [14]. Profiling circulating miRNAs can be used as a biomarker for the health of various organs and can offer valuable information about the physiological condition of an organism. This can be helpful in detecting diseases at an early stage [6, 7, 15].

Numerous investigations have supported the utilization of circulating miRNA levels as potential biomarkers for diagnosing diabetes and its consequences [14, 16]. In individuals with type 2 diabetes, a reduction in the amount of miR-15a, miR-29b, miR-125b, miR-130b, miR-126, miR-192, miR-423-5p, and miR-223 in the blood, or an elevation in the amounts of miR-28-3p circulating in the body, has been documented [17, 18]. Liu et al. [19] have discovered a connection between decreased levels of miR-192 and the advancement of diabetic nephropathy. Diabetes-induced male infertility has been associated with alterations in the expression of miRNA at both systemic and tissue levels. For instance, elevated levels of miR-93, miR-320, and miR-16 in the bloodstream may be linked to erectile dysfunction in individuals with diabetes, whereas increased expression of miR-935 and miR-504 could potentially cause harm to Leydig cells in patients with type 2 diabetes [20]. Furthermore, selectively inhibiting the activity of miR-935 and miR-504 may have the ability to alleviate the testicular problems caused by high blood sugar levels in individuals with diabetes [21]. Therefore, miRNAs have attracted significant attention as possible diagnostic and therapeutic biomarkers for problems related to diabetes. We conducted a thorough investigation of the correlation between variations in tissue and circulating miRNAs in both animal and clinical studies. We also analyzed the genetic factors and biological pathways affected by these miRNAs, which have a role in the development of male reproductive problems caused by both type I and II diabetes.

Table 1 PEO definition

Patient/Population	Exposure	Outcomes
Diabetic male animals Diabetic men HG-treated testicular cells	miRNA	Association of miRNAs and their target genes with diabetes-induced male infertility

Materials and method of search

PEO development

Since our study is descriptive, we used PEO (Table 1). First, we defined the population of interest (P), exposure (E), and outcomes (O), and questions were formulated accordingly by the authors.

1) In male diabetic patients, is there an association between exposure to diabetes-induced miRNA change and male infertility?
2) In male diabetic animals, is there an association between exposure to diabetes-induced miRNA change and male infertility?

3) In high glucose-treated cells, is there an association between high glucose induced-testicular cell dysfunction?

Protocol and search strategy

The systematic review methodology adhered to the principles specified in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) checklist. In order to conduct a thorough search, relevant articles published in English were discovered and gathered from the PubMed, Google Scholar, Cochrane Library, and ScienceDirect databases. There were no restrictions on the dates for the search. The search strategy employed a comprehensive approach by utilizing a variety of phrases and keywords, such as "microRNAs, miRNA, miRNAs, diabetic reproductive complication, diabetes, diabetes mellitus, hyperglycemia, high glucose, male infertility, male fertility, male gonad, testis histology, testis morphology, testis stereology, Leydig cell, steroidogenesis, Sertoli cell, spermatogenesis, sperm, spermatozoa, sperm parameters, erectile dysfunction." The search was refined using Boolean operators (AND/OR/NOT). In order to guarantee thoroughness, the reference lists of relevant papers were carefully reviewed to find any supplementary research that might have been missed in the first search. The latest search was performed on August 23, 2023.

Eligibility criteria and data collection

The search procedure for this study was independently conducted by three authors of our team. The authors (FO, ZL, and RK) employed a thorough search strategy, including search, assessment, and selection procedures. The search procedure entailed assessing papers based on their title, abstract, and full text, as illustrated in Fig. 1, in order to locate research that satisfied the required inclusion criteria. Whenever there were disagreements, all researchers participated in consultations to achieve a consensus.

The criteria for determining eligibility for article selection were as follows: 1). The articles must be original published studies that specifically investigate miRNAs linked to male infertility in humans or animals with either type I or II diabetes mellitus. 2) The articles must be written in English. 3) The articles must provide clear descriptions of the methods used to detect and measure miRNA. In contrast, papers were not included if they: (1) addressed other forms of diabetes, (2) were pre-prints or unpublished research, or (3) were non-original pieces such as reviews, conference articles, case reports, editorials, book chapters, or commentaries. In addition, articles were rejected if they primarily focused on the treatment of the diabetes group with miRNA mimic or miRNA inhibitors, without including a diabetic control group that experienced reproductive difficulties.

Quality assessment of selected studies

The quality of study was assessed utilizing the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Prevalence Studies. A score beyond 70% signifies great quality and low risk, while a score between 50% and 70% indicates moderate risk and medium quality. A score below 50% suggests low quality and high risk [22].

Results

Search results and included studies

As illustrated in Figs. 1 and 1989 studies were obtained from multiple databases and following the elimination based on the inclusion/exclusion criteria a total of 20 studies were included in the current review. Among the 20 studies, 12 were carried out on rats [23–34], two on mice [35, 36], two on humans [20, 37], two on both rats and humans [21, 38], one on mouse GC-1 spg cells subjected to hyperglycemia (a condition related to diabetes) [39], and one study on both rats and cell culture [40]. The studies were conducted on type I diabetes (14 studies) [24–35, 38, 40], type II diabetes (5 studies) [20, 21, 23, 36, 37], and in vitro diabetic state (one research) [39]. The investigations were conducted between 2015 and 2023.

The levels of miRNA expression were measured in serum ($n=4$) [20, 21, 37, 38], testis tissue or testicular cells ($n=8$) [23, 28–32, 35, 40], corpus cavernosum or penis ($n=7$) [24–27, 33, 34, 36], and mouse GC-spg ($n=1$) [39]. Aberrant expression profiles of 25 miRNAs were identified in diabetes associated with male reproductive issues, with 15 miRNAs exhibiting increased expression and 10 miRNAs showing decreased expression. The miRNAs that were increased in expression levels were miR-1306-5p [40], miR-935 [21], miR-504 [21], miR-484 [21], miR-320 [20], miR-206 [36], miR-205 [25], miR146a [32], miR-93 [20], miR-92a [33], miR-34a [23, 35], miR-34b [28, 29], miR-18a [36], miR-16 [20], and hsa-let-7E-5p [37]. Conversely, the miRNAs that were significantly downregulated included miR-874-3p [26], miR-449a [30, 31], miR-342–3p [37], miR-328a-5p [38], hsa-miR-199b-5p [37], miR-133a [36], miR-122 [36], miR-141 [24, 34], miR-27b-3p [39], and hsa-miR-21-5p [27]. The dysregulated miRNAs were linked to testicular injury, disrupted steroid production, decreased sperm development and quality, and erectile dysfunction. Table 2 presents a concise overview of the publication details, including first author and year of publication,

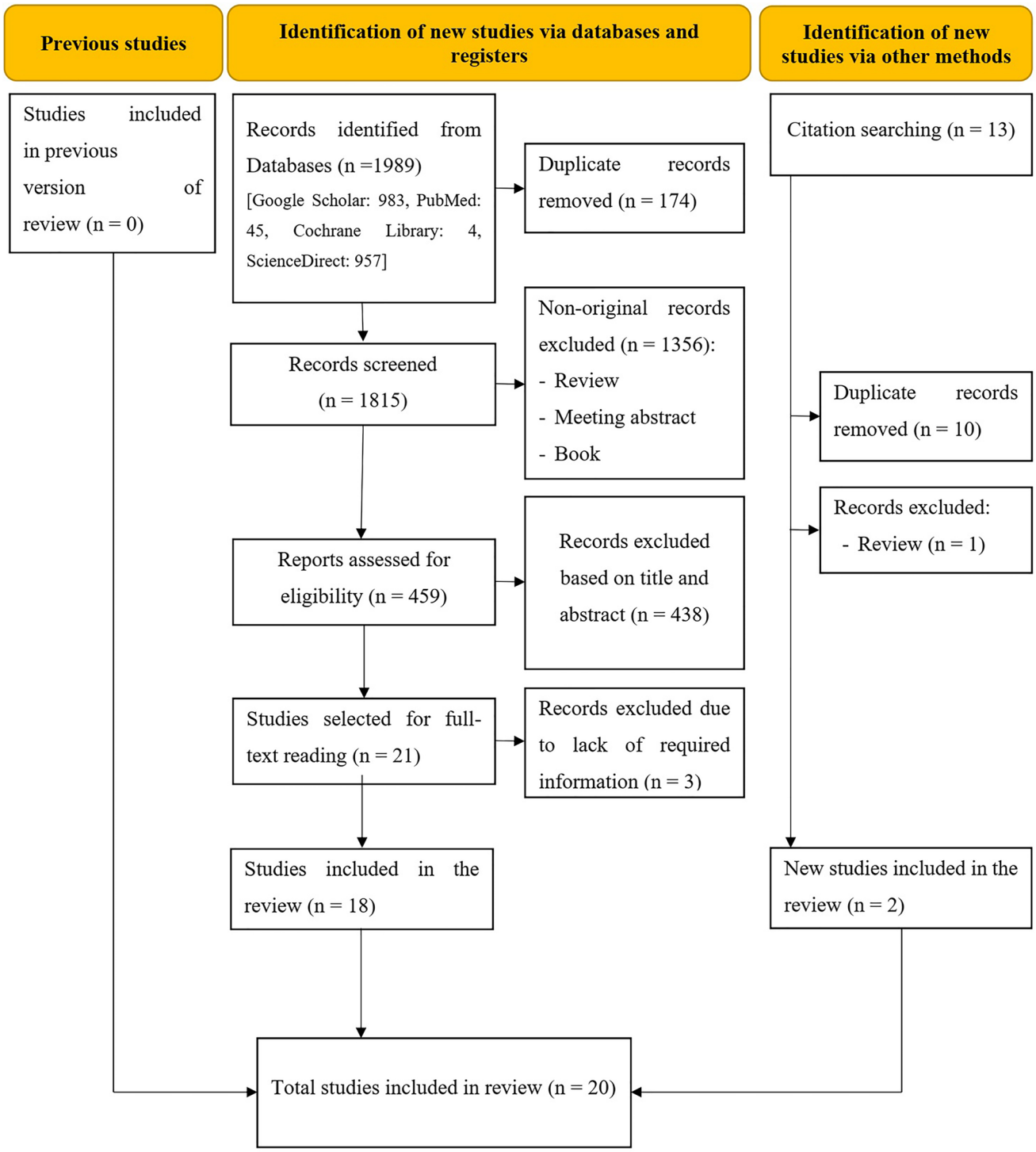

Fig. 1 Flow diagram of the literature search procedure

population (human or animal or in vitro sample), type of diabetes, miRNA analysis (sample type, expression pattern, and targeted gene), and outcomes of the included studies.

Quality of selected studies

Among the chosen publications, eighteen were identified as low-risk studies, whilst two were classed as moderate quality. All data were included regardless of the quality of the

Table 2 Characteristics of the studies included in the review

Study	Population/ Type of diabetes	Evaluated sample	miRNA alteration	Targeted gene	Outcome
Hu et al. 2021	Sprague–Dawley rats and human/ Type II diabetes	Leydig cells (rats)/ Serum (human)	• Upregulation of miR-484 (only in human), miR-504, and miR-935	• Downregulation of MEK5-ERK5-MEF2C	- Increase: Number of apoptotic sperm and somatic cells - Decrease: Testicular index, testosterone levels, spermatogenesis, proliferation rate of R2C and Leydig cells
Jiao et al. 2018	C57BL/6 mice/ Type I diabetes	Testicular cell	• Upregulation of miR-34a	• Downregulation of SIRT1	- Increase: Testicular oxidative stress (ROS and MDA), endoplasmic reticulum stress (CHOP and BIP proteins), apoptotic cell death (Bax to Bcl2 ratio, p-caspase 3, and c-caspase 3) - Decrease: Spermatozoa count, SIRT1 protein
Gaderpour et al. 2020	Wistar rats/Type 2 diabetes	Testicular cell	• Upregulation of miR-34a	• Downregulation of SIRT1	- Increase: Testicular acetylated P53, MDA, and immotile sperm - Decrease: Testicular GPx, SOD, and catalase activities, SIRT1 expression, sperm count, motility, viability, and morphology
Jiang et al. 2015	Human/ Type 2 diabetes	Serum	• Upregulation of miR-93, miR-320, and • miR-16	-	- Decrease: IIEF-5 score, serum total testosterone levels
Wang et al. 2022	Sprague Dawley rats and HG-treated Leydig cells/ Type 1 diabetes	Testis	• Upregulation of miR-1306-5p	• 17β-hydroxysteroid dehydrogenase (HSD17B7)	- Increase: MDA and ROS, testicular protein of CHOP and Grp78, acetylated P53, MDA, and immotile sperm - Decrease: Testicular weight, testicular and serum level of testosterone and SOD activity, testicular expression of StAR, p450scc, 3βHSD, 17βHSD, and HSD17B7 - Activation: Janus kinase 2/signal transducer and activator of transcription 3 axis
Wen et al. 2019	Sprague Dawlay rats/Type 1 diabetes	Cavernous bodies	• Upregulation of miR-205	• Downregulation of androgen receptor	- Increase: Expression of Caspase-3 and Bax in cavernous bodies - Decrease: Androgen receptor and Bcl-2 expression, smooth muscle density in cavernous bodies, erections and fibrosis of corpus cavernosum
Huo et al. 2019	Sprague-Dawley rats and human with erectile dysfunction/Type 1 diabetes	Smooth muscle of penis (rats)/ Serum (human)	• Downregulation of miR-328a-5p	• Upregulation of lipoprotein lipase	- Increase: Expression of lncRNA-MIAT and LPL in VSMCs and smooth muscle of rat penis and human serum, serum ROS levels, vascular endothelial cells damage, cleaved caspase-3 and Bax protein levels in vascular endothelial cells - Decrease: Serum levels of NO and GSH, expression of PECAM-1 and eNOS in rats penis corpus cavernosum, proliferation of vascular endothelial cells, erectile frequency and ICP/MAP ratio
Pan et al. 2016	db/db and wild-type mice/ Type 2 diabetes	Corpus cavernosum	• Upregulation of miR-18a and miR-206 • Downregulation of miR-122 and miR-133a	• Downregulation of 28 different genes especially *IGF-1*	- Increase: Apoptosis and fibrosis in corpus cavernosum - Decrease: ICP and ICP/MAP, percentage of smooth muscle and endothelium within corpus cavernosum
Huo et al. 2020	Sprague-Dawley rat/Type 1 diabetes	Corpus cavernosum	• Downregulation of miR-874-3p	• Upregulation of Nupr1/Chop	- Increase: Apoptosis of cavernosum smooth muscle cells, TBARS, p53, and caspase3, Nupr1, and Chop - Decrease: ICP/MAP, erection time

Table 2 (continued)

Study	Population/ Type of diabetes	Evaluated sample	miRNA alteration	Targeted gene	Outcome
Oghbaei et al. 2018	Wistar rats/Type 1 diabetes	Testis	• Upregulation of miR-34b	• Upregulation of p53	- Increase: Testes structural disorders (interstitial space, vascular congestion, destruction of germinal epithelium of seminiferous tubule, and many vacuoles in the germinal epithelium), impaired testicular morphology - Decrease: serum level of NOx, testosterone, and insulin, tubule differentiation index, spermiogenesis index, and repopulation index, sperm count, viability, motility, and percentage of spermatozoa with normal morphology and normal DNA
Keyhanmanesh et al. 2019	Wistar rats/Type 1 diabetes	Testis	• Downregulation of miR-449a	• Upregulation of p53, Pdcd4, and Pacs2	- Increase: Dead spermatozoa, sperm deformity index, spermatozoa with damaged DNA and sperm malformations, apoptotic germinal cells and tubular apoptosis index - Decrease: Sperm motility, Sertoli and Leydig cells, spermatogonia, spermatocytes, round and elongated spermatids
Keyhanmanesh et al. 2018	Wistar rats/Type 1 diabetes	Testis	• Downregulation of miR-449a	• Upregulation of p53, Pdcd4, and Pacs2	- Increase: Dead sperm, sperm deformity index, sperm DNA fragmentation sperm malformations and immotile sperm, testis tubular apoptosis - Decrease: sperm motility, number of Sertoli and Leydig cells, spermatogonia, spermatocytes, round and elongated spermatids
Oghbaei et al. 2019	Wistar rats/Type 1 diabetes	Testis	• Upregulation of miR-34b	• Upregulation of p53	- Increase: Teratozoospermia index, structural disturbance in testis - Decrease: Tubule differentiation index, spermiogenesis index, repopulation index, total sperm count, viability, motility, testosterone level
Zheng et al. 2022	Mouse GC-1 spg cells treated with 30 mmol/L D-glucose For 24 h	Mouse GC-1 spg cells	• Downregulation of miR-27b-3p	• Upregulation of Gfpt1	- Increase: Oxidative stress (ROS and MDA), inflammation (TNF-α, IL-6, and IL-1β), autophagy, apoptosis (Bax, cleaved caspase-3 and Bcl-2) - Decrease: Activity of SOD, cell viability, activation of HBP signaling
Naderi et al. 2022	Rats/ Type 1 diabetes	Testis	• Upregulation of miR146a	• Upregulation of TLR4 and IRAK1 • Downregulation of TRAF6	- Increase: Testicular TNF-α, IL1β, TLR4, and IRAK1 proteins, germ cell depletion, edema, and germ cell dissociation - Decrease: Height of the epithelial layer and the tubular diameter, spermiogenesis index, tubular differentiation index, testicular expression of TRAF6 and NF-κB
Tang et al. 2022	Sprague-Dawley rats/Type 1 diabetes	Corpus cavernosum	• Upregulation of miR-92a	• Downregulation of Prkaa2	- Increase: Oxidative stress, β-actin and MDA - Decrease: Endothelial cell proliferation, endothelial cell-cell junction protein, SOD activity, ICP/MAP and total ICP, eNOS, p-eNOS and relative ratio of p-eNOS/eNOS, occludin, VE-cadherin, and claudin-5, claudin-5/-actin, occludin/-actin, and claudin-5/-actin, HO-1, eNOS/NO/cGMP signaling

Table 2 (continued)

Study	Population/ Type of diabetes	Evaluated sample	miRNA alteration	Targeted gene	Outcome
Xu et al. 2021	Human with erectile dysfunction/ Type 2 Diabetes	Serum	• Upregulation of hsa-let-7E-5p • Downregulation of hsa-miR-199b-5p and hsa-miR-342–3p	• 40 mRNA • 9 mRNA • 15 mRNA	- Decrease: IIEF-5 score
Huo et al. 2020	Sprague-Dawley rats with erectile dysfunction/Type 1 diabetes	Corpus cavernosum	• Downregulation of miR-21-5p	• Upregulation of Pdcd4	- Increase: Apoptosis of corpus cavernosum smooth muscle cells, Pdcd4 expression - Decrease: erection frequency, ICP and MAP, proliferation of corpus cavernosum smooth muscle cells, α-SMA area and smooth muscle/ collagen in corpus cavernosum
Wen et al. 2019	Sprague-Dawley rats with erectile dysfunction/Type 1 diabetes	Penile tissues	• Downregulation of miR-141	• Upregulation of NGFRAP1	- Increase: collagen deposition, protein levels of NGFRAP1, NGF, and p75NTR in penile tissue - Decrease: NGF/p75NTR signaling, content and density of corpus cavernosum smooth muscle, ICP/MAP, α-SMA and desmin
Zhang et al. 2018	Sprague-Dawley rats/Type 1 diabetes	Penile cavernous smooth muscle cells	• Downregulation of miR-141	• Upregulation of Rho	- Increase: collagen fiber density, penile vascular wall thickness, stromal component, RhoA and ROCK2 proteins - Decrease: number of smooth muscles and irregular lumen, size of penis, number of sinusoids

MEK5: MAPK/ERK kinase 5; ERK5: extracellular signal-regulated kinase 5; MEF2C: myocyte enhancer factor 2 C; SIRT1: sirtuin; TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP nickend labeling; Bax: Bcl-2-associated X protein; Bcl2: B-cell lymphoma 2; CHOP: C-EBP homologous protein; BIP: binding immunoglobulin protein; ER stress: endoplasmic reticulum stress; ROS: reactive oxygen species; MDA: malondialdehyde; GPx: glutathione peroxidase; IIEF-5: international index of erectile function − 5; StAR: steroidogenic acute regulatory protein; 3βHSD: 3β-hydroxysteroid dehydrogenase; 17βHSD: 17β-hydroxysteroid dehydrogenase; Grp78: glucose-regulated 78; LPL: lipoprotein lipase; VSMCs: vascular smooth muscle cells; ICP/MAP: intracavernous pressure/mean arterial pressure; NO: nitric oxide; GSH: glutathione; eNOS: endothelial nitric oxide synthase; PECAM-1: platelet and endothelial cell adhesion molecule 1; TBARS: thiobarbituric acid reactive substances; Nupr1: nuclear protein-1; NOx: nitrogen oxides; Pdcd4: programmed cell death 4; Pacs2: phosphofurin acidic cluster sorting protein 2; HBP: hexosamine biosynthetic pathway; Gfpt1: glutamine–fructose-6-phosphate transaminase 1; TLR4: toll-like receptor 4; TRAF6: tumor necrosis factor receptor-associated factor 6; IRAK1: interleukin-1 receptor-associated kinase 1; HG: high glucose; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-α: tumor necrosis factor alpha; IL1β: interleukin-1 beta; p-eNOS: phosphorylated endothelial nitric oxide synthase; SOD: superoxide dismutase; HO-1: heme oxygenase 1; α-SMA: alpha smooth muscle actin; NGFRAP1: nerve growth factor receptor-associated protein 1; NGF/p75NTR: nerve growth factor/ p75 neurotrophin receptor; ROCK: Rho-associated coiled-coil containing kinases

study. Consult Table 3 for a comprehensive evaluation of the chosen studies' quality.

MicroRNAs associated with diabetes-induced testicular damage

Among the 25 miRNAs linked to male reproductive difficulties in diabetes, miR-1306-5p [40], miR-935 [21], miR-504 [21], miR-484 [21], miR-449a [30, 31], miR146a [32], miR-34a [23, 35], and miR-34b [28, 29] have been identified as contributors to testicular injury in diabetes. Of these, a change of miR-34 has been consistently detected in four investigations [23, 28, 29, 35]. A further study provided evidence that miR-34a triggers cell death in the testes by reducing the activity of sirtuin 1 (SIRT1) and elevating the levels of acetylated P53 [41]. Likewise, elevated levels of miR-34b in the testes were observed to reduce the tubule differentiation index and disturb the structure of the testis [29]. Another crucial function of increasing the expression of miR-34a in diabetes is the control of apoptosis triggered by oxidative stress [23]. Like miR-34b, miR-34a also has the ability to target SIRT1 [42]. Elevated expression of miR-504 and miR-935 in the testes of rats, as well as miR-935, miR-504, and miR-484in the blood of diabetic humans,

Table 3 Quality assessment according to the Joanna Briggs Institute critical appraisal tool for prevalence studies ($n=20$)

	Criteria	Response options		
1	Was the sample frame appropriate to address the target population?	Yes	No	Cannot Determine/ Not Applicable/ Not Reported
2	Were study participants sampled in an appropriate way?	Yes	No	Cannot Determine/ Not Applicable/ Not Reported
3	Was the sample size adequate?	Yes	No	Cannot Determine/ Not Applicable/ Not Reported
4	Were the study subjects and the setting described in detail?	Yes	No	Cannot Determine/ Not Applicable/ Not Reported
5	Was the data analysis conducted with sufficient coverage of the identified sample?	Yes	No	Cannot Determine/ Not Applicable/ Not Reported
6	Were valid methods used for the identification of the condition?	Yes	No	Cannot Determine/ Not Applicable/ Not Reported
7	Was the condition measured in a standard, reliable way for all participants?	Yes	No	Cannot Determine/ Not Applicable/ Not Reported
8	Was there appropriate statistical analysis?	Yes	No	Cannot Determine/ Not Applicable/ Not Reported
9	Was the response rate adequate, and if not, was the low response rate managed appropriately?	Yes	No	Cannot Determine/ Not Applicable/ Not Reported

Quality Rating											
Studies	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total (% of "yes")**	**Overall rating**
Jiang, 2015	Y	U	Y	N	Y	Y	U	Y	Y	66.67	Moderate
Pan,2016	Y	Y	Y	Y	Y	Y	Y	N	Y	88.89	Low
Oghbaei, 2018	Y	Y	Y	Y	Y	Y	Y	Y	N	88.89	Low
Jiao, 2018	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	Low
Keyhanmanesh, 2018	Y	Y	Y	Y	Y	N	U	Y	Y	77.78	Low
Keyhanmanesh, 2019	Y	Y	Y	N	Y	Y	Y	N	Y	77.78	Low
Wen, 2019	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	Low
Huo, 2019	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	Low
Huo, 2020	Y	Y	Y	N	Y	N	U	Y	U	55.55	Moderate
Oghbaei, 2020	Y	Y	Y	N	Y	Y	U	Y	Y	77.78	Low
Gaderpour, 2021	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	Low
Hu, 2021	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	Low
Wang, 2022	Y	Y	Y	Y	Y	Y	Y	N	Y	88.89	Low
Zheng, 2022	Y	Y	Y	Y	Y	N	U	Y	Y	77.78	Low
Naderi, 2023	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	Low
Zhang, 2018	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	Low
Wen, 2019	Y	U	Y	N	Y	Y	Y	Y	Y	77.78	Low
Tang, 2023	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	Low
Xu, 2021	Y	Y	U	Y	Y	Y	Y	Y	Y	88.89	Low
Huo, 2020	Y	Y	Y	Y	Y	Y	Y	Y	Y	100	Low

N, no; U, unclear; Y, yes, NA: Not applicable

Rater authors: FO, AF, ZL

The National Institutes of Health (NIH) quality assessment tool for observational cohort and cross-sectional studies was used (Website: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools)

has been demonstrated to reduce the testicular index and enhance Leydig cell death [21]. In addition, it was discovered that elevated levels of miR-1306-5p in the testicular tissue of diabetic rats and in Leydig cells exposed to high glucose stimulate the process of testicular cell death [40]. Previous studies have demonstrated that decreased levels of miR-449a in the testes of diabetic rats resulted in a reduction in Leydig and Sertoli cells, an increase in apoptotic germinal cells, and an enhanced tubular apoptosis index [30, 31]. Also, a recent study has found that elevated levels of miR-146a play a role in apoptosis and inflammation in the tissues of the testes [32].

MicroRNAs associated with diabetes-induced steroidogenesis destruction

Several distinct miRNAs have been discovered as exhibiting an influence on the process of gonadal steroidogenesis. The following microRNAs have been linked to this process: miR-1306-5p [40], miR-935 [21], miR-504 [21], miR-484 [21], miR-449a [30, 31], and miR-34b [28, 29]. Increased expression of miR-935 and miR-504 was seen in the Leydig cells of rats with diabetes produced by streptozotocin (STZ), and also elevated levels of miR-935, miR-504, and miR-484 in serum samples from humans who have type 2 diabetes. The higher levels of miRNA were discovered to reduce both the amounts of testosterone in the bloodstream and in the testes by triggering apoptosis in Leydig cells [21]. Additionally, it was found that elevated levels of miR-1306-5p in the testicular tissue of rats with diabetes produced by STZ hindered the production of steroids by specifically targeting important enzymes involved in steroidogenesis [40]. Furthermore, previous research revealed that the disruption of testicular miR-34b in diabetes induced by STZ for a duration of two to three months was associated with a decline in testosterone levels and the quantity of Leydig cells [29]. The findings emphasize the pivotal role of miRNAs in controlling testosterone synthesis and the possible consequences for diseases like diabetes.

Dysregulated miRNAs in diabetes-induced spermatogenesis impairment

Multiple studies have provided evidence of the role of miRNAs in defining different sperm characteristics, such as sperm count, motility, and morphology [43]. Studies have discovered several miRNAs that are dysregulated in diabetes and have a direct effect on sperm parameters. Testicular samples from diabetic animals or individuals have shown an increase in the expression of miR-935 [21], miR-504 [21], miR-484 [21], miR146a [32], miR-34a [23, 35], and miR-34b [28, 29]. In contrast, the decrease in expression of miR-449a [30] and miR-27b-3p [39] has been found to impact sperm characteristics in individuals with diabetes.

In a study conducted by Hu et al. [21], it was shown that increased levels of miR-504 and miR-935 in diabetes resulted in reduced sperm production in the seminiferous tubules and a higher proportion of sperm cells undergoing apoptosis. In addition, the upregulation of miR-34a in the testicular tissue of male Wistar rats with type II diabetes was linked to a higher percentage of immotile sperm, as well as decreased sperm count, motility, viability, and normal morphological criteria [23]. Furthermore, the increased expression of miR-34b in the testicular tissue of rats with diabetes induced by STZ led to a reduction in the spermiogenesis index, sperm count, viability, motility, and the proportion of spermatozoa with normal morphology and DNA [35].

The decreased expression of miR-449a in diabetic rats was linked to several negative effects on sperm quality, including a higher percentage of dead spermatozoa, an elevated sperm deformity index, damaged DNA in spermatozoa, abnormal sperm morphology, and apoptotic germinal cells. Additionally, there was a decrease in sperm motility, as well as reductions in spermatogonia, spermatocytes, and round and elongated spermatids [30, 31]. The study conducted by Zheng et al. [39] revealed a considerable downregulation of miR-27b-3p in mouse GC-1 spg cells, which are immortalized cells generated from the testis of adult mice and closely represent a stage between type B spermatogonia and primary spermatocytes. This downregulation occurred when the cells were exposed to a high concentration of glucose. This downregulation led to elevated oxidative stress and inflammation, reduced cell viability, and triggered autophagy and apoptosis. Nevertheless, the excessive expression of miR-27b-3p effectively neutralized these changes by inhibiting the activation of the hexosamine biosynthesis pathway signaling in spermatogenic cells treated with high glucose [39]. Furthermore, an increase in the expression of miR-146a was observed in testicular tissue samples taken from Wistar rats with type 1 diabetes caused by STZ. This increase in miR-146a expression was found to be linked to a decrease in the spermiogenesis index [32]. To summarize, the disruption of certain miRNAs, including miR-935, miR-504, miR-484, miR-146a, miR-34a, miR-34b, miR-449a, and miR-27b-3p, in diabetes has an impact on several sperm characteristics and the processes involved in spermatogenesis.

Dysregulated miRNAs in diabetes-induced erectile dysfunction

Individuals with diabetes mellitus have a significantly higher occurrence of sexual dysfunctions, such as erectile problems compared to those without diabetes. This prevalence is six times more in diabetic patients [44, 45]. MiRNAs have been

discovered to have a vital role in the development of erectile dysfunction caused by diabetes [46]. Studies conducted on animals and humans with diabetes have revealed an increase in the expression of hsa-let-7E-5p [46], miR-16 [20], miR-18a [36], miR-92a [33], miR-93 [20], miR-205 [24], miR-206 [36], miR-320 [20], and a decrease in the expression of miR-21-5p [27], miR-122 [36], miR-133a [36], miR-141 [24, 34], hsa-miR-199b-5p [37], miR-328a-5p [38], hsa-miR-342–3p [37], and miR-874-3p [26] in the serum or corpus cavernosum. These changes have been associated with erectile dysfunction caused by diabetes. For example, two studies have demonstrated that downregulated miR-141 in penile tissues of STZ-induced diabetes caused structural and functional alterations in the corpus cavernosum and a decrease in intracavernous pressure (ICP)/mean arterial pressure (MAP) leading to erectile [24, 34]. A clinical investigation by Jiang et al. [20] found elevated blood levels of miR-320, miR-93, and miR-16 in diabetes patients with erectile dysfunction, suggesting that the serum levels of these miRNAs may be crucial for the diagnosis of diabetic erectile dysfunction. Furthermore, elevated miR-205 in the corpus cavernosum of STZ-induced diabetic rats indicated abnormalities in the cavernous bodies, including decreased smooth muscle density and androgen receptor, raised apoptosis, fibrosis of corpus cavernosum, and lowered number of erections [25]. Elevated levels of miR-18a and miR-206, along with reduced levels of miR-122 and miR-133a in the corpus cavernosum of type 2 diabetic db/db mice resulted in a decline in erectile function parameters (ICP/MAP) and a decrease in the percentage of smooth muscle cells and endothelium within the corpus cavernosum [36].

The downregulation of miR-328a-5p has been associated with vascular endothelial cell damage, reduced frequency of erections, and alterations in ICP/MAP. This has been observed in both the vascular smooth muscle cells of diabetic Sprague-Dawley rats and the serum of diabetes patients with erectile dysfunction [38]. This downregulation also resulted in decreased expression of platelet and endothelial cell adhesion molecule 1 (PECAM-1) and endothelial nitric oxide synthase (eNOS) in the corpus cavernosum of diabetic rats, as well as elevated levels of reactive oxygen species (ROS) and decreased nitric oxide (NO) and glutathione (GSH) levels in the serum of diabetic patients [38].

MiR-874-3p, a noteworthy microRNA, has been found to decrease in the corpus cavernosum of STZ-induced diabetic rats. This decrease leads to a reduction in ICP/MAP and erection time, as well as an increase in cellular death of cavernosum smooth muscle cells. These effects are mediated by the enhancement of thiobarbituric acid reactive substances (TBARS), p53, and caspase3 in the corpus cavernosum [26]. Two distinct experiments conducted on the corpus cavernosum of rats with STZ-induced diabetes revealed that the decrease in miR-141 resulted in a reduction in the quantity of corpus cavernosum sinusoids, the content and density of corpus cavernosum smooth muscle, and the irregular size of the penis's lumen. Additionally, it affected the ICP/MAP, as well as the positive rate of smooth muscle actin (α-SMA) and desmin. Furthermore, the presence of collagen and its concentration in the penile corpora cavernosum were elevated, the supportive tissue component was strengthened, and the wall of the penile blood vessels became thicker by specifically targeting nerve growth factor receptor-associated protein 1 (*NGFRAP1*) and *Rho* genes [24, 34].

Diabetes-induced upregulation of miR-92a resulted in a decrease in endothelial cell proliferation, expression of proteins involved in endothelial cell-cell junctions, max ICP/MAP and total ICP, levels of occludin, claudin-5, and vascular endothelial-cadherin (VE-cadherin), superoxide dismutase (SOD) activity, expression of heme oxygenase-1 (HO-1), inhibition of eNOS/NO/cyclic guanosine monophosphate (cGMP) pathway, and an elevation in the relative expression of β-actin and malondialdehyde (MDA) level pathway in the corpus cavernosum. According to the authors' conclusion, miR-92a specifically affects the protein kinase AMP-activated catalytic subunit alpha 2 (*Prkaa2)* gene in the corpus cavernosum [33]. The inhibition of miR-21-5p in smooth muscle cells of the corpus cavernosum, both in vitro using a high glucose medium and in diabetic rats with erectile dysfunction, led to a decrease in the frequency of erections, ICP/MAP levels, α-SMA area, and an increase in apoptosis of the corpus cavernosum. Additionally, there was a reduction in the proliferation of smooth muscle/collagen and the overall smooth muscle content in the corpus cavernosum smooth muscle cells. MiR-21-5p specifically targeted the Bax/bcl2/caspase3 signaling pathway through downregulation of *Pdcd4* gene in the corpus cavernosum, as indicated by a study [27]. In fact, miR-21-5p suppresses apoptosis of corpus cavernosum smooth muscle cells (CCSMCs) by targeting PDCD4 in diabetic rats.

Discussion

This study is the inaugural systematic evaluation investigating alterations in miRNA expressions in the serum and assessments of both diabetic animals and humans with male infertility [1]. Bioinformatic analyses have shown that differently expression of specific miRNAs and their target genes in STZ-induced diabetic rats lead to male subfertility. Figure 2 illustrates the complete set of signaling pathways that are influenced by modified miRNAs in diabetes, resulting in male reproductive problems. Twenty studies have documented alterations in 25 miRNAs in the serum, semen,

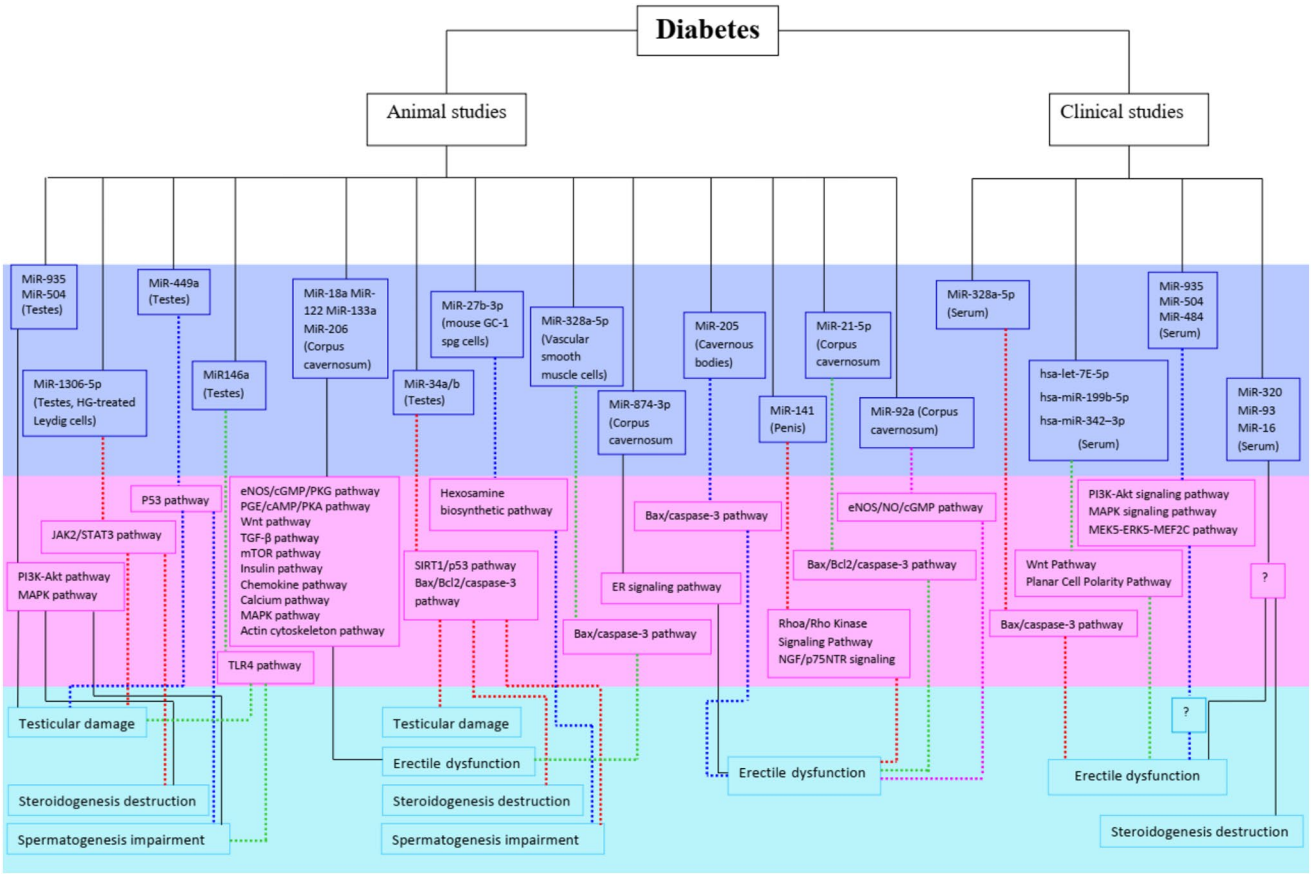

Fig. 2 Signaling pathways dysregulated by altered miRNAs expression in diabetes-induced male reproductive complications. Each line with specific color is only for identifying each miRNA and its target signaling pathway. Blue section shows altered miRNAs, pink section shows signaling pathways, and green section shows complications

or testes of patients with diabetes, which have been linked to male reproductive issues such as damage to the structure and function of the testes, impaired sperm production, and sexual behavioral problems.

Hyperglycemia and insulin shortage are recognized factors that contribute to changes in miRNA expression, resulting in damage to the testes and impaired spermatogenesis in males with diabetes-related reproductive issues [21, 28–31]. Hyperglycemia in diabetes significantly contributes to the excessive production of miR-935 and miR-504 in the testes of diabetic rats, as well as in the blood of young male patients with type II diabetes and cultured Leydig tumor R2C cells. The combined effect of elevated levels of miR-935 and miR-504 is a reduction in the growth rate of Leydig cells and the production of testosterone, while also increasing the rate of apoptosis through the inhibition of the mitogen/extracellular signal-regulated kinase kinase-5 (MEK5)-extracellular signal-regulated kinase 5 (ERK5)-myocyte enhancing factor-2 C (MEF2C) pathway [21]. The MEK5-ERK5-MEF2C pathway plays a role in promoting cell proliferation and preventing cell death [47, 48]. Suppression of the MEK5-ERK5-MEF2C pathway and testicular damage generated by high glucose can be reversed by specifically inhibiting miR-935 and miR-504, as demonstrated in a study [21]. MiR-1306-5p is an additional microRNA that is influenced by high blood sugar levels. Elevated expression of miR-1306-5p in Leydig cells treated with high glucose or in the testes of diabetic rats generated by STZ leads to a reduction in testosterone output and an increase in cell death. This effect is achieved by suppressing the activity of hydroxysteroid 17-beta dehydrogenase 7 (HSD17β7), as indicated by previous research [40]. Reducing the activity of HSD17β7 increases apoptosis via a decrease in the quantities of glucose-regulated 78 (Grp78) proteins, eIF2α phosphorylation, and C/EBP homologous protein (Chop) [40]. Nevertheless, the overexpression of HSD17β7 counteracts the reduction in testosterone output and suppresses the cell death caused by miR-1306-5p overexpression in Leydig cells or testes of diabetic rats induced by STZ through the activation of the janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway. Oxidative damage to the testis is mediated by the JAK2/STAT3 signaling pathway [49]. The mechanism by which HSD17β7 prevents testosterone reduction in Leydig cell is due to it plays an essential role

in cholesterol biosynthesis. HSD17B7 can perform multiple conversions within cholesterol synthesis including the conversion of lanosterol to sterol, estrone into estradiol, and zymosterone into zymosterol [50, 51]. Moreover, HSD17B7 convert androstenedione to testosterone in mice [52]. These findings indicate that HSD17B7 is an enzyme that plays a role in cholesterol and testosterone biosynthesis.

The occurrence of testicular cell apoptosis in diabetes, as a result of high blood sugar levels (hyperglycemia), is partially influenced by the miR-449/phosphofurin acidic cluster sorting protein 2 (Pacs2)/ programmed cell death protein 4 (Pdcd4) and miR-34/SIRT-1 pathways [30, 31]. MiR-449 exhibits a high level of expression in healthy testes and governs cellular apoptosis by processes that can be either dependent on or independent of p53, as indicated by previous studies [53, 54]. The decreased expression of miR-449a and the increased expression of miR-34b were linked to an elevated occurrence of cell death in the testes of diabetic rats induced by STZ. This effect was achieved by targeting the genes *p53*, *SIRT1*, *Pacs2*, and *Pdcd4* [23, 28–31]. MiR-34a results in a reduction of SIRT1 protein [55], thus leading to an elevation in acetylated p53 [55]. Consequently, the activation of p53 leads to an increase in the transcription of the miR-34a gene [56], which in turn leads to a decrease in the levels of the SIRT1 protein through a positive feedback loop. SIRT1 inhibits apoptosis by inhibiting the activation of p53 [57]. The targeted suppression of miR-34a and the selective activation of SIRT1 reduced the diabetes-induced apoptotic cell death in the testes of male mice with diabetes (C57BL/6) [35]. Studies have demonstrated that insulin therapy can effectively prevent and correct alterations in the expression of miR-449a and miR-34b, as well as their target genes [28–31]. MiR-146a, a microRNA, plays a role in causing inflammation and programmed cell death in the testicles of individuals with diabetes. It achieves this by targeting the toll like receptor 4 (TLR4)/interleukin-1 receptor-associated kinase 1 (IRAK1)/TNF receptor-associated factor 6 (TRAF6) signaling pathway [32]. Elevated levels of miR-146a were observed in the testes of diabetic rats, leading to histological alterations such as depletion and dissociation of germ cells, edema, as well as reduced height of the epithelial layer and tubular diameter [38]. Nevertheless, the authors did not validate their findings by employing knockdown or inhibition of miR-146a or its associated pathways, thereby leaving their conclusions unconfirmed [38].

Glucose regulates the biological processes of germ cells via influencing the expression of miRNA in a manner that depends on the dosage. Research has demonstrated that exposing GC-1 spermatogenic cells to elevated glucose levels leads to the suppression of miR-27b-3p [39]. The viability of GC-1 spermatogenic cells is reduced by downregulating or silencing miR-27b-3p, which leads to an increase in oxidative stress and inflammation, triggers autophagy, and facilitates apoptosis. Nevertheless, this harmful impact was counteracted when miR-27b-3p was excessively produced or when Glutamine-Fructose-6-Phosphate Transaminase 1 (Gfpt1) was suppressed [39]. Research indicates that miR-27b-3p may target the Gfpt1/hexosamine biosynthesis pathway (HBP) axis [39]. Suppression of miR-27b-3p results in an upregulation of Gfpt1 expression, which serves as the enzyme that limits the rate of the HBP [39]. The excessive activation of the HBP is recognized as a crucial element in facilitating the progression of diabetes and its associated problems [58, 59]. The HBP can be activated by hyperglycemia and insulin resistance, both of which are variables linked to diabetes [60]. The activation of the HBP can lead to increased oxidative stress and trigger cell death in hyperglycemic hearts [61]. Downregulation of Gfpt1 has been demonstrated to mitigate the deleterious impact of miR-27b-3p suppression on spermatogenic cell damage generated by high glucose, and relieve the injury in spermatogenic cells treated with high glucose [39].

In male mice with diabetes, researchers discovered that by specifically blocking miR-34a (miR-34a-I) and activating SIRT1 using a SIRT1-selective activator (SRT2104), they were able to maintain an optimal sperm count [35]. This suggests that miR-34 is an important microRNA for normal sperm production [62]. Studies have demonstrated that elevated levels of SIRT1 protein can reduce sperm abnormalities and DNA damage caused by diabetes [63]. SIRT1 also affects spermatogenesis process and spermatogenic stem cells, as well as germ cell function. In this line, Coussens et al. have reported that SIRT1 deficiency attenuates spermatogenesis, spermatogenic precursors, the proportion of mature sperm with increased DNA damage, and numbers of mature sperm in SIRT1−/− mice [64]. Microarray analysis in SIRT1 deficient testis showed dysregulated expression of 85 genes involved in spermatogenesis [64]. SIRT1 is well identified for its nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase function. By its deacetylase activity, SIRT1 regulates the hepatic metabolism of glucose, insulin secretion from β cells, and the insulin signaling pathway (reviewed by liang et al. [65]). SIRT1 enhances insulin secretion of pancreatic beta cells in response to glucose stress by suppressing the expression of UCP2 [66, 67]. UCP2 regulates insulin secretion and glucose metabolism by reducing NADH levels, weakening mitochondrial membrane potential, decreasing ATP production, and suppressing the generation of superoxide [68–70]. SIRT1 also impacts a crucial step in the insulin signaling pathway via deacetylation of insulin-induced tyrosine phosphorylation of insulin receptor substrate-2 (IRS-2) [71]. Srt1720, a SIRT1 activator, suppresses insulin-induced hepatic gluconeogenesis in mice and obese rats through

deacetylase activity of SIRT1 [72, 73]. Liver-specific knockdown of SIRT1 augmented insulin sensitivity and systemic glucose [74]. In addition, the absence of miR-34b/c and miR-449 in the testes results in infertility since it causes the generation of atypical spermatozoa with decreased motility. The absence of miR-34 and miR-449 hinders the last phases of sperm maturation by impeding miosis [53]. The exact procedure via which diabetes affects the expression of miR-34a and miR-449a is not fully comprehended, although it is hypothesized that increased oxidative stress and apoptosis are the primary routes implicated [30, 31, 35, 41].

Diabetic males frequently experience erectile dysfunction, with a prevalence rate of 85% [75]. Although the precise reason for erectile dysfunction in diabetes is not completely understood, diabetic angiopathy has been recognized as a notable contributing component [20, 76]. MiRNAs have a significant impact on the regulation of angiogenesis by influencing many pathways and proteins that are involved in the function of endothelial cells, including eNOS and NO [77, 78]. NO, generated by the action of NO synthase (NOS) enzymes using L-arginine as a substrate, induces the relaxation of smooth muscle cells in the corpora cavernosa, leading to the occurrence of penile erection [79]. Diabetes-induced hyperglycemia results in an excessive generation of oxidative stress, which leads to the excessive utilization of NO and thus causes erectile dysfunction [80, 81]. The current emphasis in medical treatments for erectile dysfunction is on maximizing the internal signaling of NO. An example of this is when the miR-92a antagomir was injected into the cavernous body, it enhanced the ability to achieve an erection in rats with diabetes by activating the eNOS/NO/cGMP and adenosine monophosphate-activated protein kinase (AMPK)/eNOS signaling pathways. The administration of this therapy led to upregulation of VE-cadherin, occludin, claudin-5, HO-1, eNOS, and p-eNOS, while simultaneously reducing ICP/MAP and total ICP. Furthermore, it was observed that miR-92a exhibited an elevation in cavernous endothelial cells in a laboratory setting simulating diabetes circumstances, resulting in the suppression of Prkaa2 [33]. Prkaa2 is a subject of regulation by miR-92a and plays a role in the AMPK/eNOS and AMPK/nuclear factor erythroid 2–related factor 2 (Nrf2)/HO-1 signaling pathways, which are essential for preserving oxidative equilibrium and endothelial function [33]. Other microRNAs, including miR-18a, miR-122, miR-133a, and miR-206, contribute to endothelial function by selectively regulating several genes and pathways, such as eNOS/cGMP/PKG and insulin-like growth factor 1 (*IGF-1*) [36]. *IGF-1*, which is regulated by miR-18a and miR-206, has been observed to decrease in the corpus cavernosum of both diabetic animals and humans suffering from erectile dysfunction [36, 82]. IGF-1 has a crucial role in the restoration of NOS in the intracavernosal nerves [83]. In addition, a reduction in the levels of eNOS and endothelial cell adhesion molecule 1 has been noticed in the smooth muscle cells of the penis and vascular smooth muscle cells that were grown in high-glucose conditions after the overproduction of long non-coding RNAs-myocardial infarction-associated transcript (lncRNA-MIAT) caused a decline in miR-328a-5p [38]. The overexpression of lncRNA-MIAT results in increased production of lipoprotein lipase (LPL), leading to vascular endothelial cell (VEC) damage and programmed cell death by decreasing serum levels of GSH and NO, as well as reducing eNOS expression. Additionally, it causes an elevation in ROS levels, cleaved caspase-3, and Bax expression [38]. Upregulating miR-328a-5p or downregulating lncRNA-MIAT can provide protection against erectile dysfunction by enhancing the production of eNOS in VECs and reversing the decrease in ICP/MAP and erectile frequency [38]. It is important to mention that an excessive amount of LPL has been associated with the occurrence of erectile dysfunction [84]. Furthermore, miR-93 and miR-125b have also been associated with erectile function [38]. MiR-93 has the potential to impact erection by modifying the levels of vascular endothelial growth factor, whereas miR-125b has the potential to enhance the production of inflammatory genes in vascular endothelial cells [85, 86]. The function of the cavernous nerve is equally important to the function of the endothelium in the process of achieving an erection. Research has demonstrated that the application of miR-92a antagomir can partially revive the expression of neuronal NOS (nNOS) in the corpus cavernosum of diabetic rats experiencing erectile dysfunction. This indicates that miR-92a antagomir has a neuroprotective impact on the function of the cavernous nerve [33]. The miR-874-3p/Nuclear protein 1(Nupr1)/Chop pathway is another pathway that is involved in erectile dysfunction caused by vascular problems such as inflammation and apoptosis of vascular endothelial cells in diabetes [26, 87]. MiR-874-3p forms a bond with Nupr1 and hinders its activity, resulting in a pro-apoptotic impact [87, 88]. Nupr1 upregulates Chop expression, perhaps contributing to vascular diseases through its involvement in endoplasmic reticulum stress-induced apoptosis in individuals with diabetes and erectile dysfunction [89, 90]. Suppression of Nupr1 also amplifies the expression of antioxidants [91]. Thus, miR-874-3p has an antioxidant effect by inhibiting the Nupr1/Chop pathway [26]. The decrease in miR-874-3p in rats with diabetes leads to the stimulation of apoptosis in cavernosum smooth muscle cells and the development of erectile dysfunction via raising the expression of Nupr1. The reversal of this effect can be achieved by increasing the expression of miR-874-3p and suppressing the activity of Nupr1 [26]. Additionally, a reduction in the growth rate and an increase in the

programmed cell death of smooth muscle cells in the corpus cavernosum, which were obtained from the erectile tissue of the penis and cultured in a high glucose condition, were observed. This effect was also observed in diabetic rats with erectile dysfunction. The changes were attributed to the downregulation of miR-21-5p and the upregulation of Pdcd4 in the cavernous tissue [27]. The researchers stated that the introduction of mesenchymal stromal/stem cell (MSC)-exosomes harboring miR-21-5p into CCSMCs, or the suppression of Pdcd4, leads to an increase in CCSMC population and a reduction in CCSMC apoptosis, resulting in an enhancement of erectile function. This emphasizes the possible involvement of the miR-21-Pdcd4 axis in combating erectile dysfunction caused by diabetes by suppressing apoptosis in CCSMCs [27]. It has been reported that Pdcd4 suppresses apoptosis via inhibiting the procaspase-3 mRNA translation, however loss of Pdcd4 through the specific RNA interference enhanced procaspase-3 expression leading to its activation and PARP cleavage and sensitized the cells to apoptosis [92]. MicroRNA-141 is a significant miRNA that controls the rate at which cells in the corpus cavernosum divide and undergo programmed cell death. The presence of lower levels of microRNA-141 in the smooth muscle cells of the penile cavernous area in rats with diabetic erectile dysfunction leads to an increased rate of cell growth and a reduced rate of cell death in the corpus cavernosum. This is achieved by targeting the Rho-GTPase A (RhoA)/ Rho-associated kinase 2 (ROCK2) and nerve growth factor receptor (NGF)/p75 neurotrophin receptor (p75NTR) signaling pathways [24, 34]. Rho-associated proteins are linked to the production of reactive oxygen species, modification of the cytoskeletal actin structure, cell growth, and specialization [93]. ROCK2 acts as the downstream mediator of RhoA and enhances cell motility by inhibiting myosin phosphatase [94, 95]. The utilization of MiR-141 mimics and ROCK inhibitor (ripasudil) results in a reduction in apoptotic rates and an enhancement of erectile function in diabetic rats [96]. Additionally, research has demonstrated that the RhoA/ROCK signaling pathway plays a role in sustaining an erection in a NO-independent manner [97]. The expression of miR-141 is increased and the expression of NGFRAP1 is decreased in order to improve ICP, MAP, and erectile function in diabetic rats with erectile dysfunction. This is achieved by suppressing the NGF/p75NTR signaling pathway [24]. NGFRAP1 controls the process of cell death triggered by p75NTR [98]. The androgen receptors (AR) located in the cavernous bodies have been identified as having a crucial function in the process of achieving an erection [99]. In rats with diabetes and erectile dysfunction, the increased expression of miR-205 negatively affects the androgen receptor in the cavernous bodies [25]. As a consequence, there is a reduction in the expression of AR, leading to inadequate blood flow in the penile arteries, which is a major factor contributing to erectile dysfunction [100]. In order to tackle this problem, scientists have discovered that reducing the activity of miR-205 and/or increasing the expression of AR can boost the growth and hinder the programmed cell death of CSMCs. This ultimately leads to an improvement in erectile function in diabetic rats with erectile dysfunction by preventing the formation of fibrous tissue in the corpus cavernosum. When the miR-205 inhibitor was administered, it resulted in an increase in ICP, MAP, ICP/MAP ratios, and the activation of the AR. Additionally, it led to a decrease in the formation of new capillaries in the corpus cavernosum tissues [25]. Although obtaining tissue samples through biopsy is necessary for evaluating miRNA expression in humans, it is important to recognize that alterations in miRNA expression in tissues can also be identified in bodily fluids. Measuring the serum level of miRNAs is a more appropriate method for detecting and predicting the disease complications. Jiang et al. [20] discovered a substantial elevation in the levels of miR-16, miR-93, and miR-320 in the blood of diabetes patients with erectile dysfunction. This finding suggests that measuring the serum concentrations of these miRNAs could be valuable for diagnosing erectile dysfunction in individuals with diabetes. In a study conducted by Xu et al. [37], it was shown that the serum of 10 diabetic patients with erectile dysfunction showed elevated levels of hsa-let-7e-5p and reduced levels of hsa-miR-199b-5p and hsa-miR-342–3p. These microRNAs were discovered to specifically target 40, 9, and 15 messenger RNAs, respectively. Additionally, they had a negative correlation with the IIEF-5 (international index of erectile function − 5 score). Furthermore, a negative association was seen between IIEF-5 and testosterone, glycated globin, and fasting blood glucose [37]. Moreover, a reduced concentration of miR-328a-5p in the blood has been documented in individuals with diabetes who experience erectile dysfunction [38]. Notably, the dysregulated miRNAs discussed in this review, namely miR-16, miR-21, miR-27, miR-34, miR-122, miR-141, miR-146, miR-449, and miR-504, were also observed to be dysregulated in cases of infertility unrelated to diabetes [53, 101–108]. These nine miRNAs may play a significant effect in male fertility. Only miR-16 has been observed in the blood of diabetic patients with erectile dysfunction, as seen from a clinical standpoint. Further research is required to examine the modification of additional miRNAs in the blood of male diabetic patients experiencing reproductive difficulties.

The most important limitation of our study was the incapacity to do a meta-analysis. The main reason for this was a lack of adequate raw data regarding the relative expression or fold changes of miRNAs. Moreover, the existence of methodological and technical differences among research,

as well as variations in miRNA quantification methods, posed additional obstacles to our capacity to carry out a meta-analysis.

Conclusions

This review highlights 25 dysregulated miRNAs in the testes or serum of individuals with diabetes who experience reproductive difficulties. This systematic study stands out for its exhaustive review of dysregulated miRNAs in the blood and testes of those with diabetes, encompassing both animal and clinical investigations. This is the first time such a comprehensive report has been compiled. The results demonstrate that the miRNA–mRNA network is linked to the pathological progression of diabetic testicular damage or erectile dysfunction. From a therapeutic perspective, the identification of circulating miRNAs could be beneficial in the timely identification and prevention of diabetes problems, such as diabetes-induced male infertility. By identifying and focusing on specific dysregulated miRNAs in both serum and testes, it is possible to develop new diagnostic and treatment strategies for male infertility caused by diabetes. Among all signaling pathways influenced by modified miRNAs, the Bax-caspase-3, mitogen-activated protein kinases (MAPK), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), and eNOS-cGMP-protein kinase C (PKC) were the main deregulated pathways, thus they might be potential pathways for diagnosis and therapy of diabetes-induced male infertility. Future research should prioritize the examination of the precise miRNAs, as well as their corresponding target genes and pathways, which contribute to the development and advancement of male infertility caused by diabetes. This will enhance our comprehension of the fundamental mechanisms and maybe discover new treatment targets. Although significant advancements have been achieved in the identification of dysregulated miRNAs in diabetes, there remains a considerable number of miRNAs yet to be discovered, particularly in diabetic individuals' serum. These miRNAs hold potential in the diagnosis and treatment of male reproductive issues associated with diabetes. Additional examination of the dysregulated miRNAs and their functional roles will yield useful insights into the development of diabetes-induced male infertility and maybe facilitate the creation of targeted therapies. Additional investigation is necessary to examine the miRNA–mRNA network and its significance for diagnosing and treating male reproductive problems associated with diabetes.

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Declarations

Ethics approval and consent to participate Not applicable.

Consent to publication Not applicable.

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Authors and Affiliations

Zeinab Latifi[1] · Saba Nikanfar[2] · Rasa Khodavirdilou[3] · Sohrab Minaei Beirami[4,5] · Lida Khodavirdilou[3] · Amir Fattahi[6] · Farnaz Oghbaei[7]

✉ Amir Fattahi
amirfattahi@gmail.com

✉ Farnaz Oghbaei
hoghbaei1988@gmail.com

Zeinab Latifi
zeinablatifi@yahoo.com

Saba Nikanfar
saba.nikanfar@uclouvain.be

Rasa Khodavirdilou
Rasa.khodavirdilou@ttuhsc.edu

Sohrab Minaei Beirami
minaei.sohrab@yahoo.com

Lida Khodavirdilou
lidakhodavirdilou@gmail.com

[1] Nervous System Stem Cells Research Center, Semnan University of Medical Sciences, Semnan, Iran

[2] Pôle de Recherche en Physiopathologie de la Reproduction, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium

[3] Department of Pharmaceutical Sciences, Jerry H. Hodge School of Pharmacy, Texas Tech University Health Sciences Center (TTUHSC), Amarillo, TX, USA

[4] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

[5] Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

[6] Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[7] Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran