

# The Emerging Role of MicroRNAs in Female Reproductive Diseases

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## ABSTRACT

Female infertility represents a significant global medical concern, attributable to a spectrum of disorders affecting the reproductive system, which encompasses premature ovarian insufficiency (POI), polycystic ovary syndrome (PCOS), Asherman syndrome (AS), endometriosis, preeclampsia, ovarian cancer, and endometrial cancer. It exerts a pervasive impact on women's reproductive well-being on a global scale. Recent years have witnessed a mounting body of evidence, drawing from investigations employing murine models and human clinical data, which elucidates the dysfunction of microRNAs (miRNAs) in various reproductive pathologies. This dysfunction, it appears, plays a pivotal role in the context of female gametogenesis and fertility. Nonetheless, the precise mechanistic links between miRNA dysfunction and the pathogenesis of these disorders remain, in many cases, inadequately understood. This comprehensive review endeavors to shed light on recent advancements in research pertaining to the regulatory functions of miRNAs in the etiology of diverse reproductive disorders that culminate in infertility. Furthermore, it addresses the potential utility of miRNAs as diagnostic biomarkers and therapeutic targets for these conditions. The paper also examines the existing limitations and challenges inherent to the clinical application of miRNAs in this context.

## 1. Introduction

Infertility is characterized by the inability to achieve a clinical pregnancy after one year or more of unprotected and frequent intercourse [1, 2]. Among infertility cases, one-third can be attributed to female factors. Reproductive system-related disorders stand as the primary culprits for female infertility [3], encompassing ovulatory dysfunction, tubal infertility, endometriosis, as well as uterine and cervical factors [4]. These disorders pose a significant threat to the reproductive health of women and are a major contributor to infertility [2, 4]. As a result, they have garnered substantial attention from clinicians and researchers, who are committed to the development of novel therapeutic approaches to

prevent and treat infertility, ultimately enhancing the quality of life for patients and their partners. However, despite these efforts, there have been limited recent advancements in the diagnosis and treatment of female reproductive disorders. Various therapeutic modalities, including pharmaceutical interventions, surgical procedures, and assisted reproductive technology (ART), have been employed to restore female fertility. Hormone replacement therapy (HRT) is commonly administered in clinical settings to address reproductive disorders [5]. Nevertheless, the extended use of HRT entails an elevated risk of diverse complications, including cardiovascular disease and cancer [6, 7]. While ART has been instrumental in the treatment of infertility and has significantly improved pregnancy outcomes for infertile couples, its overall success rates remain relatively low, especially for women of advanced maternal age. Additionally, ART procedures are highly invasive and linked to medical complications such as hyperstimulation syndrome [8, 9]. Therefore, it is imperative to explore more advanced treatment strategies.

Often, infertility and pregnancy loss can be directly attributed to ovarian aging. With increasing age, there is a marked decline in the quantity of follicles and oocytes, collectively referred to as the “ovarian reserve”, which results in infertility. Investigating the various pathophysiological mechanisms underlying female reproductive disorders may provide specific targets for effective infertility treatment and the prevention of long-term complications in this patient population. Recent studies have suggested that noncoding RNAs may play a role in regulating the pathophysiology of female reproductive disorders [10-12], offering a potential avenue for novel treatment strategies. Over the past decade, a growing body of evidence has shown that noncoding RNAs, recognized as pivotal post-transcriptional regulators in various diseases, can also govern the physiological processes of sex steroid biosynthesis and secretion [10, 13]. Furthermore, an expanding body of clinical and translational research has emerged, shedding new light on the foundational research in this field. Among noncoding RNAs, microRNAs (miRNAs) have been the subject of extensive study. Their regulatory influence in ovarian cells has been thoroughly investigated, revealing their impact on processes such as steroidogenesis, gonadal development, ovulation, apoptosis, and corpus luteum development [12]. Nevertheless, the potential relationship between miRNAs and female reproductive disorders, including premature ovarian insufficiency (POI), polycystic ovary syndrome (PCOS), Asherman syndrome (AS), endometriosis, preeclampsia, ovarian cancer, and endometrial cancer,

remains to be fully elucidated, and clinical management of female reproductive disorders continues to present significant challenges.

In this review, we focus on recent research progress regarding the role of differentially expressed miRNAs in the pathogenesis of female reproductive disorders. Additionally, we explore their potential as biomarkers of these disorders and as targets for therapeutic interventions, with the objective of elucidating the molecular mechanisms through which miRNAs contribute to the development of female reproductive disorders. We will also address the persisting limitations and issues that require further investigation. A comprehensive understanding of the role of miRNAs in female reproductive disorders may provide valuable insights and guidance for improving female fertility, reproductive quality, and the maintenance of female reproductive health.

## 2. Biological Properties of miRNAs

It is noteworthy that the extensively investigated protein-coding genes constitute a mere 1.5% of the entire genome [14]. The term “non-coding RNA” pertains to RNA molecules that do not encode proteins but exert critical regulatory functions in the realms of development, physiology, and diseases [15]. This category has thus garnered increasing attention. Among these non-coding RNAs, miRNAs stand as the most extensively studied class. miRNAs typically comprise 21-22 nucleotides and can mediate the post-translational gene silencing, thereby suppressing protein expression [16, 17]. miRNAs exert their regulatory influence by recognizing the 3' untranslated region (3' UTR) of the target gene through their initial 8 residues at the 5' end, known as the “seed sequence”. Subsequently, they engage in Watson-Crick base pairing to impede transcription [18, 19]. It is important to note that miRNAs are evolutionarily conserved and exhibit tissue-specific expression patterns [20]. The expression and functional role of miRNAs are contingent upon specific cell types. Remarkably, miRNAs have been identified as pivotal regulators of mammalian follicular cell physiology, ovarian function and oocyte maturation, through regulating the expression of fertility-related genes. An accumulating body of research indicates that miRNAs are closely related to various female reproductive disorders, including POI[21], PCOS[22], AS[23], endometriosis[24], preeclampsia[25], ovarian cancer[26], and endometrial cancer[27]. The potential utility of miRNAs in the pathology and management of these diseases has been established. Nonetheless, the precise underlying mechanisms remain shrouded in obscurity.

### 3. The Role of miRNAs in the Pathophysiology of Female Reproduction

#### 3.1 Premature Ovarian Insufficiency

Premature ovarian insufficiency (POI), also known as premature ovarian failure (POF), is characterized by the cessation of menstruation before the anticipated onset of menopause [28]. POI represents a leading cause of female infertility, marked by elevated gonadotropin levels and diminished estrogen levels, often accompanied by primary or secondary amenorrhea [29]. This condition gives rise to short term complications such as menopausal symptoms and exerts long-term effects on bone health, cardiovascular health, cognitive function, fertility, and sexual function [30, 31]. Notably, the prevalence of POI is rising among women of reproductive age, affecting approximately 1 in 100 women under the age of 40 [32]. The progression from ovarian insufficiency to full menopause can take months or even years, and exposure to gonadotoxic agents, such as chemotherapy, may expedite ovarian failure [33]. The disorder is highly heterogeneous, and abnormal follicular development at all stages may contribute to POI [34]. There is a close relationship between ovarian granulosa cells (GCs) quality and the occurrence of POI because senescence and cell cycle disorders in GCs result in a significant reduction of ovarian reserve [35-37]. The occurrence of POI can result from an insufficient primordial follicle pool reserve, accelerated follicular atresia, changes in dominant follicle recruitment, and follicular maturation disorders [38, 39]. The etiology of POI is notably multifaceted, with contributions from genetic factors [40], autoimmune [41, 42] and endocrine disorders, mitochondrial dysfunction, pharmaceutical agents, surgical interventions, environmental factors, and psychological stressors [43]. Nevertheless, a substantial portion of the etiological factors remains enigmatic.

Most available studies on the association between miRNA and POI have been conducted in murine models, focusing on miRNA profiles as it relates to aberrant ovarian follicle growth. Several miRNAs have been identified as dysregulated in POI, although mechanistic data is far more limited. In a study involving a mouse model exposed to a high-fat and high-sugar (HFHS) diet, known to induce oxidative stress and pose a risk for various diseases, the mice exhibited the typical pathological characteristics of POF. However, treatment with poly (lactic-co-glycolic acid) (PLGA)-cross-linked miR-146b-5p nanoparticles (miR-146@PLGA) resulted in downregulated p38-Mapk14 expression in GCs, alleviated GCs ageing, and mitigated POF symptoms [44]. Another investigation unveiled that exosomal miR-122-5p promoted the apoptosis of ovarian

GCs by targeting BCL9 in a POI mouse model induced by cyclophosphamide and busulfan. This suggested that miR-122-5p holds potential as a target for restoring ovarian function [45]. Furthermore, the therapeutic effects of thymopentin were demonstrated through the transcriptional activation of Lin28A and the inhibition of let-7 family microRNAs, thus ameliorating the ageing of ovarian GCs and effectively treating POF in a HFHS diet-induced mouse model [46]. Research efforts have also delved into the relationship between miRNA expression and the pathogenesis of autoimmune POI. Notably, miR-21 has emerged as a key player in ovarian folliculogenesis, with its low expression levels correlated with autoimmune POI in both mouse models induced by ZP3 immunization and in patients. This indicates a potential role for miR-21 in the pathogenesis of autoimmune POI [47]. Furthermore, studies have unveiled the miRNAs profile and functional relevance in biochemical POI, revealing the presence of potentially pathogenic miR-379-5p and identifying two novel targets PARP1 and XRCC6, which corroborated the significance of DNA repair for POI [48].

In mammals, the primordial follicles serve as the ovarian reserve. The decline in ovarian function with aging is characterized by a gradual reduction in both the quantity and quality of the oocytes residing within these primordial follicles. Research involving exosomes derived from human umbilical cord mesenchymal stem cells (HucMSC-exos) has demonstrated their specific accumulation in primordial oocytes and their ability to restore ovarian function in POI or naturally aging animal models. These effects are attributed to the activation of the oocyte PI3K/mTOR signaling pathway and the acceleration of follicular development, facilitated by functional microRNAs, such as miR-146a-5p or miR-21-5p [49]. Another study showcased the restoration of ovarian phenotype and function in a POI mouse model through HucMSC-exos. These exosomes promoted the proliferation of CTX-damaged GCs and ovarian cells while mitigating ROS accumulation by delivering exosomal miR-17-5p, which suppressed PARP1,  $\gamma$ H2AX, and XRCC6 by inhibiting SIRT7. This work highlights the essential role of exosomal miR-17-5p and its downstream target mRNA, SIRT7, in the context of HucMSC-exos transplantation therapy, offering potential insights for miRNA-based POI treatment [50]. Additionally, another study revealed that miR-126-3p, overexpressed in HucMSC, promoted angiogenesis and attenuated ovarian GCs apoptosis in POI, suggesting the potential of exosomes containing miR-126-3p as an effective therapeutic strategy for POI treatment [51]. Collectively, these findings propose miRNAs as a novel approach to ameliorate age-related fertility decline in women, providing an epigenetic perspective on the disease.

### 3.2 Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) represents a highly prevalent endocrine disorder afflicting women of reproductive age and serves as a common etiology for menstrual irregularities and infertility [52]. Clinical manifestations of PCOS encompass hyperandrogenemia, polycystic ovarian morphology, ovulatory dysfunction, menorrhagia, and hyperinsulinemia owing to insulin resistance (IR). The presence of hyperandrogenemia is intricately linked with various comorbidities related to PCOS, including anovulation and metabolic repercussions [53]. PCOS affects up to 20% of women of reproductive age and is intricately associated with obesity, insulin resistance, dyslipidemia, type II diabetes, and, potentially, cardiovascular events [54]. This high prevalence has positioned PCOS as the most common endocrine disorder affecting women of reproductive age. Existing treatments for PCOS encompass lifestyle modifications (such as dietary and exercise interventions, often recommended as first-line therapies in international evidence-based guidelines), pharmacological approaches (including insulin sensitizers and combined oral contraceptive pills), as well as invasive interventions (such as assisted reproductive techniques and artificial insemination), all aimed at enhancing various aspects of health outcomes [55]. Lifestyle modifications and complementary and alternative medicines often take precedence as initial therapeutic strategies. Despite mounting evidence indicating that PCOS may be a complex multigenic disorder influenced by robust epigenetic and environmental factors, including dietary and lifestyle elements, the precise etiology and pathophysiology remain incompletely understood. Furthermore, the condition lacks universally accepted diagnostic biomarkers and targeted therapy options.

To elucidate the role of miRNAs in the progression of PCOS, numerous studies have been conducted on human samples and various experimental models. For instance, one study revealed that miR-93-5p, which has been documented as elevated in GCs of PCOS patients, promotes apoptosis and ferroptosis in GCs by modulating the NF- $\kappa$ B signaling pathway [56]. Simultaneously, researchers investigated the molecular mechanism of miR-96-5p in the context of PCOS and its potential applications. Elevated circulating miR-96-5p were significantly correlated with the disordered endocrine clinical features of PCOS, demonstrating promising diagnostic potential with an area under the receiver operating characteristic curve of 0.8344, alongside 75.71% specificity and 80% sensitivity. Mechanically, miR-96-5p was identified as an androgen-regulated miRNA that

directly targets the forkhead transcription factor FOXO1 [57]. Another study conducted by Zhang et al. assessed the diagnostic, therapeutic, and functional potential of miR-335-5p, which was recently discovered and observed to be reduced in the follicular fluid of PCOS patients compared to healthy women. Circulating miR-335-5p levels were found to significantly correlate with impaired endocrine and clinical features associated with PCOS in human patients [58]. More recently, exosomes released by adipose mesenchymal stem cells were identified as inhibiting the expression of B-cell translocation gene 2 through the transfer of miR-21-5p to the livers of rats with PCOS. This action activated the IRS1/AKT pathway and improved hepatic metabolism, providing protection against metabolic disturbances, ameliorating ovarian polycystic features, and enhancing fertility [59]. Extracellular vesicles-miR-26b derived from adipocytes were also shown to inhibit viability and promote apoptosis in cumulus cells, thereby disrupting ovary ovulation in the context of PCOS by targeting JAG1 [60]. Another study elucidated that miR-21 regulates GCs apoptosis and proliferation in PCOS by targeting toll-like receptor 8 [61]. These findings offer compelling evidence that miRNA may serve as mediators in the etiopathogenesis of PCOS, potentially fulfilling roles as novel diagnostic biomarkers and therapeutic avenues for PCOS. Considering the limited number of completed clinical trials, which often have small sample sizes and frequently lack conclusive results regarding repurposed medications for PCOS, further well-designed clinical research in this area would be advantageous.

### 3.3 Asherman Syndrome

Asherman syndrome (AS) represents an acquired clinical condition characterized by severe clinical symptoms that significantly impair reproductive function. Common manifestations include intrauterine adhesion (IUA), menstrual disturbance (amenorrhea), infertility, and recurrent pregnancy loss [62]. These adhesions develop following uterine surgery or dilation and curettage procedures, with more complex intrauterine surgeries performed using advanced technology increasing the risk of AS. The formation and progression of AS involve endometrial fibrosis. The scar tissue or adhesions formed within the uterine cavity and/or cervical canal hinder blastocyst implantation, disrupt blood supply to the uterus and early fetus, ultimately resulting in recurrent miscarriages or infertility [63]. Additionally, patients with recurrent AS often exhibit a thin endometrium [64]. It is estimated that over 90% of AS cases develop after curettage associated with pregnancy. Notably, the prevalence of AS has grown in tandem with the increased



rates of cesarean and endometrial surgeries. Despite several treatment options, including hysteroscopic interventions considered the gold standard for diagnosis and treatment, hyaluronic acid gel, hormone therapy, uterine perfusion, and amniotic membrane transplantation, the management of AS remains challenging [65]. The complexity of the syndrome can lead to complications such as uterine perforation, bleeding, infection, and, in some cases, early miscarriage following procedures. Strategies to minimize risk and reduce its severity are urgently needed.

Emerging research suggests that miRNAs play a pivotal role in the regulation of fibrosis and could offer potential benefits in treating AS. For instance, miR-122 expression was found to be reduced in patients with IUA, often accompanied by fibrosis. Overexpression of miR-122 reduced the extent of fibrosis in endometrial stromal cells, promoting endometrial regeneration and restoring pregnancy capacity in a mouse model of endometrial injury. Further molecular analyses revealed that miR-122 inhibited fibrosis through the TGF- $\beta$ /SMAD pathway by directly targeting the 3' untranslated region of SMAD family member 3 (Smad3), thereby suppressing its expression [23]. Another study reported that miR-326 was downregulated in endometrial tissues from patients with IUA, and its overexpression inhibited endometrial fibrosis by downregulating pro-fibrotic genes. Additionally, miR-326 overexpression blocked the activation of the TGF- $\beta$ 1/Smad3 signaling pathway by suppressing the expression of TGF- $\beta$ 1, an crucial pro-fibrogenic mediator that was identified as a direct target of miR-326 [66]. These findings indicated that miR-326 inhibited endometrial fibrosis by suppressing the TGF- $\beta$ 1/Smad3 signaling pathway, suggesting that miR-326 could serve as a prognostic biomarker and therapeutic target for IUA.

There is growing evidence that human umbilical cord mesenchymal stem cells (UCMSCs) play a vital role in repairing damaged endometrium in IUA. Furthermore, exomes released by UCMSCs can facilitate intrauterine communication by delivering miRNAs. For instance, miR-543 levels were found to be reduced in IUA tissues. UCMSCs-derived exosomal miR-543 demonstrated the ability to prevent endometrial fibrosis both *in vitro* and *in vivo* by downregulating N-cadherin [67]. Additionally, UCMSCs-derived exosomal miR-202-3p was shown to regulate the expression of MMP11 and promote the accumulation of extracellular matrix components such as COL1A1, COL3A1, COLVI, and FN in the early stages of endometrial injury repair [68]. Another study indicated that overexpression of miR-455-5p in UCMSCs helped attenuate endometrial

injury and repair the damaged endometrium by activating the SOCS3-mediated JAK/STAT3 signaling pathway [69]. UCMSCs-derived exosomal miR-145-5p ameliorated TGF- $\beta$ 1-induced endometrial fibrosis, with ZEB2 identified as a direct target inversely regulated by exosomal miR-145-5p [70]. Collectively, these findings offer valuable insights for potential clinical treatments for AS.

### 3.4 Endometriosis

Endometriosis is a prevalent multifactorial gynecological condition, reliant on estrogen and characterized by chronic inflammation, marked by the presence of endometriotic lesions located outside the uterine cavity. It affects approximately 10% of women of reproductive age [71, 72]. Notable consequences of this disorder include pelvic pain and infertility [73]. Despite the availability of various treatment options, including surgical interventions and assisted reproductive technologies, a high rate of symptom recurrence persists. Additionally, the lack of effective tools for early-stage diagnosis and treatment makes the quest for new therapeutic approaches an ongoing challenge. Furthermore, the clinical management of endometriosis-related infertility is often intricate, owing to the heterogeneity of affected patients [74].

The molecular pathogenesis of endometriosis has been linked to pathological alterations in the expression of numerous genes, miRNAs, and signaling pathways, all of which play a pivotal role in the process of epithelial-mesenchymal transition (EMT). Research has suggested that the TGF- $\beta$ 1-SMAD3-ILK signaling pathway, possibly through mechanisms related to EMT, may hold significance the pathogenesis of endometriosis. Furthermore, miR-21 has been identified as a potential inhibitor of this TGF- $\beta$ 1-SMAD3-ILK axis [75]. Recent investigations have explored the expression profile of miR-542-3p and EMT markers (BMP7, SMAD4, CKH1) in matched eutopic endometrium, ectopic endometrium samples, and peripheral blood mononuclear cells from women with endometriosis (n=29) in comparison to healthy women (n=25). These studies revealed a significant negative correlation between miR-542-3p and BMP signaling genes. This suggests that miR-542-3p may have the potential to negatively regulate the BMP7-SMAD4-CKH1 signaling pathway associated with EMT [76]. Researchers have recently proposed that uterine endothelial cells promote miR-138 to induce exosome-mediated inflammation and apoptosis in endometriosis through the VEGF/NF- $\kappa$ B signaling pathway [77]. Additionally, endometriosis derived exosomal miR-301a-3p has been shown to mediate

macrophage polarization by regulating the PTEN-PI3K axis [78]. The lack of specificity in symptoms and the absence of sensitive, noninvasive diagnostic methods often result in significant delays in diagnosis, underscoring the need for diagnostic biomarkers. The correlation between circulating miRNAs and the altered inflammatory signals observed in endometriosis patients has raised the possibility of miRNAs serving as biomarkers for the disease. One prospective cohort study explored the potential use of changes in extracellular miRNA profiles in the plasma of 51 endometriosis patients compared to 41 controls, combined with clinical data, as non-invasive biomarkers for the disease. The data indicated that a distinct plasma miRNA signature is associated with endometriosis, and that has-miR-154-5p, either alone or in combination with has-miR-196b-5p, has-miR-378a-3p, and has-miR-33a-5p, in conjunction with clinical parameters such as body mass index and age, hold potential for non-invasive diagnosis of the disease [79]. Furthermore, another clinical study analyzed miRNA expression in the saliva of women with and without endometriosis and found that has-miR-135a was significantly elevated in the saliva and plasma of women with endometriosis, regardless of disease stage and menstrual cycle phase [80]. This suggests that has-miR-135a has the potential to serve as a noninvasive biomarker for endometriosis in both saliva and plasma, although further validation studies are required to determine its clinical utility as a biomarker.

### 3.5 Preeclampsia

Preeclampsia (PE) represents a pregnancy-specific multisystem complication characterized by the onset of hypertension and proteinuria, often accompanied by maternal organ dysfunction, such as liver and kidney impairment. It affects 2% to 8% of pregnancies [81, 82] and typically emerges after 20th week of pregnancy, primarily due to placental hypoxia leading to inadequate spiral-artery remodeling [83]. There is substantial evidence suggesting a heightened risk of cerebrovascular complications following delivery in women with PE during pregnancy. Moreover, PE is a serious cardiovascular ailment and a major contributor to maternal and perinatal morbidity and mortality [84]. Consequently, more in-depth investigations and innovative approaches are imperative to effectively address this intricate disease. Despite substantial research aimed at identifying preventive measures for PE, the incidence of this condition has remained relatively unaltered over the past few decades. This persistence might be attributed to an incomplete understanding of the underlying pathophysiology of PE.

At present, there is a notable absence of a clinically viable non-invasive biomarker assay for early detection, which limits the effectiveness of PE prevention and treatment strategies. Researchers have undertaken a three-phase retrospective and prospective study, encompassing discovery, training, and validation stages, involving cross-platform and multicenter cohorts. This extensive study identified five PE-associated differentially expressed miRNAs from miRNA sequencing data, with subsequent validation of two miRNAs (miR-196b and miR-584-5p) as robust biomarkers. These miRNAs were validated through association analysis with clinical characteristics and qRT-PCR in tissue specimens during the discovery phase [85]. This discovery has paved the way for the development of a novel and reliable blood-based miRNA signature, which holds promise as a clinically applicable non-invasive tool for early PE detection. The development of PE is thought to be linked to “placental dysfunction” resulting from ischemia and hypoxia, inflammatory responses, oxidative stress, and endothelial damage in the arterial vasculature during placental development. Recent research has unveiled the involvement of ferroptosis in the pathophysiological process of PE. In PE models, upregulation of miR-30b-5p plays a pivotal role in ferroptosis, by downregulating Cys2/glutamate antiporter and PAX3, subsequently reducing ferroportin 1 (an iron exporter) expression. This results in decreased glutathione (GSH) levels and increased labile Fe<sup>2+</sup>. The inhibition of miR-30b-5p expression and supplementation with ferroptosis inhibitors have shown promise in attenuating PE symptoms in rat models, thus identifying miR-30b-5p as a potential therapeutic target for PE [86]. Furthermore, it has been suggested that miR-2115-3p may interact with GOT1 mRNA to downregulate its expression, further inhibiting hypoxia-induced ferroptosis in a PE rat model established through reduced uterine perfusion pressure surgery [87].

Several studies have proposed that abnormal placental miRNA expression may be associated with PE through the regulation of trophoblast cell invasion and apoptosis in the placenta. For instance, miR-30a-3p was found to be significantly elevated in the placenta of women with PE (n=25) compared to those with normal pregnancies (n=20). It is believed to play a role in the pathogenesis of PE by targeting IGF-1, which exhibits relatively low expression levels in PE patients, thereby regulating the invasion and apoptosis of trophoblast cells [88]. Another noteworthy factor, endocrine gland-derived vascular endothelial growth factor (EG-VEGF), is crucial for facilitating trophoblast invasion in the placenta. Elevated miR-141 and miR-200a were found to inhibit the expression of EG-VEGF, resulting in disrupted downstream extracellular signal-regulated

kinase (ERK)/matrix metalloproteinase 9 signaling and cilia formation. This leads to defective trophoblast invasion. The growth of the primary cilium, which transduced ERK signaling upon EG-VEGF induction for proper trophoblast invasion, was also inhibited by miR-141 and miR-200a upregulation [89]. In another study investigating the key miRNAs involved in PE development, a high-throughput miRNA sequencing analysis was conducted on placental tissues from patients with PE and healthy controls. This was followed by the investigation of differentially expressed miRNAs and functional enrichment analysis, ultimately revealing that miR-200b-3p is upregulated in the placental tissues of patients with PE. This miRNA promotes PE development through its association with PFN2 [90].

### 3.6 Ovarian Cancer

Ovarian cancer is a prevalent and deadly malignant tumor of the female reproductive system, and it is the leading cause of gynecologic cancer-related deaths worldwide [91]. One of the major challenges in managing ovarian cancer is its insidious onset and lack of early screening methods, resulting in over 50% of patients being diagnosed at an advanced stage [92]. This delay in diagnosis, along with the lack of specific diagnostic tools, has contributed to the high mortality associated with this disease. Annually, more than 230,000 new cases of ovarian cancer are reported worldwide, resulting in more than 150,000 deaths [93]. Although primary treatments for ovarian cancer include surgery and adjuvant therapy [94], patients often experience recurrences with chemotherapeutic resistance within a few years, leading to a 5-year survival rate of less than 50%. Developing effective early screening tools is crucial for early detection and intervention in ovarian cancer.

Recognizing the challenges in treating ovarian cancer, current research efforts are deeply involved in molecular and cellular profiling. Cancer antigen 125 is the most commonly used biomarker for ovarian cancer screening, but its specificity is low, and it is not reliable for the early detection of ovarian cancer [95]. Ongoing research is focused on identifying novel biomarkers such as autoantibodies, circulating tumor DNA (ctDNA), miRNAs, and DNA methylation signatures to improve early detection methods and reduce ovarian cancer mortality [96]. One area of interest is circulating miRNAs, which play critical roles in intercellular communication. Nevertheless, the underlying mechanism is not fully elucidated.

miRNAs encapsulated within extracellular vesicles (EVs) have emerged as mediators of intercellular communication

and potential clinical biomarkers in liquid biopsy. In one study, a panel of eight miRNAs (miR-1246, miR-1290, miR-483, miR-429, miR-34b-3p, miR-34c-5p, miR-145-5p, miR-449a) was identified based on dysregulated miRNAs that overlapped in the ascites and plasma subsets. Of particular significance, miR-1246 and miR-1290, found in malignant ascites-derived EVs, were shown to promote the invasion and migration of ovarian cancer cells by regulating a common target, ROR $\alpha$  [97]. Furthermore, exosomes derived from ascites were found to transfer miR-6780b-5p to ovarian cancer cells, which promoted EMT and facilitated metastasis [98]. Exosomal miRNAs has also been recognized as a key player in tumor-associated angiogenesis. Research has explored the potential of artificially generated exosomes with overexpressed miR-92b-3p as anti-angiogenic agents, offering a novel approach for anti-angiogenic therapy in ovarian cancer [99]. Additionally, cancer-derived exosomes have been implicated in oncogenesis by manipulating infiltrating immune cells in the tumor microenvironment, with exosomal miR-1246 having a role in ovarian cancer progression and chemoresistance, offering potential therapeutic strategies [100].

Treatment with PARP inhibitors (PARPi), such as Olaparib, has emerged as a promising option for ovarian cancer patients. Research has indicated that miR-200c significantly enhances the anti-cancer efficacy of Olaparib in drug-resistant ovarian cancer cells [101]. The combination of Olaparib with miRNA-based therapy holds promise as a treatment strategy for drug-resistant ovarian cancer, offering the potential for designing precision medicine trials to optimize the clinical use of PARPi. Moreover, miR-181a has been identified as an activator of the Wnt signaling pathway, driving stemness and chemoresistance in ovarian cancer. This discovery highlights the potential of miR-181a as a novel biomarker for  $\beta$ -catenin-targeted therapy in ovarian cancer [102].

### 3.7 Endometrial Cancer

Endometrial cancer, ranking as the fourth leading cause of malignancy in the female genital tract, is responsible for approximately 76,000 deaths among women each year worldwide [103]. The incidence of endometrial cancer has been increasing globally in recent years. While early presentation with postmenopausal bleeding ensures the diagnosis of most endometrial cancer patients at an early stage and allows for curative hysterectomy, those with advanced disease face a poor prognosis [104], with survival rates declining to 15%. Surgery is recommended

as the primary therapy for endometrial cancer, but adjuvant radiotherapy and chemotherapy are also options for some patients [105]. Identifying new targets and biomarkers is crucial for managing endometrial cancer, and a deeper understanding of the genetic diversity and drivers of this complex malignancy can improve therapeutic precision at various disease stages.

Recent studies have highlighted the significant role of miRNAs in predicting the prognosis of various types of cancers. Since the discovery of miRNAs, it has become evident that miRNAs are associated with all aspects of cell function, including malignant transformation and metastasis [106]. To enhance early detection of endometrial cancer, researchers have conducted extensive studies on plasma-derived exosomal miRNAs as potential diagnostic biomarkers. In one study, miR-15a-5p, miR-106b-5p, and miR107 were identified significantly upregulated in exomes isolated from plasma samples of endometrial cancer patients compared with healthy subjects. Particularly, miR-15a-5p alone yielded an AUC value of 0.813 to distinguish EC patients with stage I from healthy subjects. The integration of miR-15a-5p and serum tumor markers (CEA and CA125) achieved a higher AUC value of 0.899[107]. This study demonstrates that plasma-derived exosomal miR-15a-5p is a promising and effective diagnostic biomarker for the early detection of endometrial cancer.

Although many miRNAs have been reported in endometrial carcinoma, most of them were identified from tumor tissues obtained at surgery or from cell lines cultured in laboratories. In order to develop a method to detect EC-specific miRNA biomarkers from liquid biopsy samples to improve the early diagnosis of EC in women, endometrial fluid samples from 82 patients were collected and processed, with 60 matched non-cancer versus endometrial carcinoma patients used in phase I and 22 in phase II[108]. Among the 14 miRNA biomarkers, three miRNAs had a consistent and substantial fold-change in upregulation (miR-429, miR-183-5p, and miR-146a-5p). Furthermore, four miRNAs (miR-378c, miR-4705, miR-1321, and miR-362-3p) were uniquely detected. Additionally, it has been demonstrated that the composition of miRNAs in uterine fluid of women with recurrent implantation failure is different from that of healthy fertile women and the dysregulated miRNAs are associated with impaired endometrial receptivity and embryo implantation[109]. Hsa-miR-486-5p (FC -20.32; P-value=0.004) and has-miR-92b-3p (FC -9.72; P-value=0.004) were successfully technically validated

with RT-PCR. However, the screening of a larger set of clinical samples was necessary to validate these early detection biomarkers for endometrial cancer.

#### 4. Conclusion and Future Perspective

Female reproductive disorders are great threats to women's physical and mental health and contribute to infertility. Remarkably, miRNAs have shown great potential for treating female infertility in various animal models and clinical studies. Herein, this review summarized miRNAs as essential regulators in the pathophysiology and potential treatment of various female reproductive disorders, including POI, PCOS, AS, endometriosis, preeclampsia, ovarian cancer, and endometrial cancer. These miRNAs play crucial roles in modulating molecular and biological pathways associated with these conditions, offering promising therapeutic potential. Research has advanced our understanding of the mechanisms by which miRNAs influence these reproductive health-related diseases. It has also opened up opportunities for the development of novel miRNA-based treatments that could improve the quality of life and reproductive health of affected women. While miRNA-based treatments are still in the early stages of pre-clinical research or very early clinical trials, they hold great promise. There are, however, several challenges and areas for future investigation. These include:

1. Safety and efficacy: Further research is needed to establish the safety and efficacy of miRNA-based treatments for female infertility. Rigorous testing, including preclinical and clinical trials, is required to ensure that these treatments are both safe and effective.
2. Target identification: Understanding the complete regulatory and functional networks of miRNAs in specific reproductive disorders is essential. Identifying the precise targets of miRNAs involved in these conditions will aid in the development of more targeted and effective treatments.
3. Disease models: Researchers need to address the discrepancies between animal models and human disease conditions to ensure that findings in animal studies can be translated effectively to human treatments.
4. Optimal delivery methods: Determining the ideal dosage and routes of miRNA delivery for therapeutic purposes is a crucial aspect of research. Innovative delivery methods, such as nanotechnology, may offer new avenues for miRNA-based treatments.



## 5. Author Contributions

Original draft preparation, M.L. and B.X.; review and editing, M.L., Y.Z. and F.W.; visualization, M.L., M.C., J.H. and F.W.. All authors have read and agreed to the published version of the manuscript.

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