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Function of MicroRNAs in Normal and Abnormal Ovarian Activities: A Review Focus on MicroRNA-21

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Abstract

Some failures in ovary function, like folliculogenesis and oogenesis, can give rise to various infertility-associated problems, including polycystic ovary syndrome (PCOS) and premature ovarian insufficiency (POI). PCOS influences 8 to 20% of women; while POI occurs in at least 1% of all women. Regrettably, the current therapies for these diseases have not sufficiently been effective, and finding a suitable strategy is still a puzzle. One of the helpful strategies for managing and treating these disorders is understanding the contributing pathogenesis and mechanisms. Recently, it has been declared that abnormal expression of microRNAs (miRNAs), as a subset of non-coding RNAs, is involved in the pathogenesis of reproductive diseases. Among the miRNAs, the roles of miRNA-21 in the pathogenesis of PCOS and POI have been highlighted in some documents; hence, the purpose of this mini-review was to summarize the evidences in conjunction with the functions of this miRNA and other effective microRNAs in the normal or abnormal functions of the ovary (i.e., PCOS and POI) with a mechanistic insight.

Keywords: MicroRNA-21, Pathogenesis, Polycystic Ovarian Syndrome, Premature Ovarian Insufficiency

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Introduction

Dysfunctions in folliculogenesis and oogenesis, like failure in the formation of steroid hormones and maturation of oocytes, lead to various ovarian diseases related to infertility, such as polycystic ovarian syndrome (PCOS) and premature ovarian insufficiency (POI) (1-3). PCOS, as a prevalent endocrinopathy in the reproductive course of women, involves in 8 to 20% of women (4). This disease can be identified by detecting two of the three properties related to the Rotterdam criteria (i.e., hyperandrogenism, polycystic ovaries, and an- or oligo-ovulation) and excluding associated diseases (e.g., Cushing's syndrome, congenital adrenal hyperplasia, hyperprolactinemia, and thyroid disease) (5, 6). Additionally, POI [another name is premature ovarian failure (POF)] influences 1% of women at young ages and it is defined by decreased estradiol (E2) expression and follicular dysplasia, in addition to increased gonadotropin expression and follicle-stimulating hormone (FSH) (7, 8).

Unfortunately, against these disorders related to folliculogenesis and oogenesis, the current therapeutic approaches have not reflected enough effectiveness; thus, discovering a therapy with high efficiency and minimum side-effects is still a challenging issue (9, 10). One of the useful ways to improve remedies and the management of diseases is knowing the pathogenic mechanisms of illnesses (11). Accumulating evidences have implicated that abnormal expression of microRNAs was linked with pathological processes of different disorders, such as reproductive diseases, metabolic disorders, cancer, cardiovascular diseases, and neurological conditions (12-16). microRNAs (miRNAs/miRs) are a subset of non-coding and single-stranded RNAs. Their lengths are approximately 18-24 nucleotides, and they can down-regulate certain gene expression in a post-transcriptional way by binding to target messenger RNA [mRNA; 3′-untranslated region (UTR)] (17, 18). The published papers have indicated that microRNAs can be

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involved in ovarian functions, e.g., endocrine function, folliculogenesis and modulation of steroidogenesis, as well as apoptosis and proliferation of granulosa cells (19, 20). Recently, the role of dysregulation of miR-21 in the pathogenic occurrences of ovarian function-associated diseases (i.e., PCOS and POI) has been highlighted (21-23). Hence, in this mini-review, we aimed to discuss and review the role of this molecule and the other involved miRNAs in normal ovarian activities and pathogenic events of the mentioned diseases.

miR-21 is one of the most frequently present miRNAs in the ovary of different species, such as mouse, sheep, porcine, and bovine (24). miR-21 is divided into two types according to its strand, including miR-21 passenger strand (miR-21-3p) and miR-21 guide strand (miR-21-5p), the latter of which is the most frequent miRNA related to the RNA-induced silencing complex in granulosa cells (25, 26). It is approved that miR-21 (miR-21-5p) has a role in oocyte maturation as well as blastocyst and embryo development. It is considerably overexpressed at the time of transition from germinal vesicle to oocytes, arrested in the metaphase II (MII) stage of the meiotic division (27-29). In the research of Wright et al. (30), the results of reverse transcription real-time quantitative polymerase chain reaction (RT-qPCR) analysis manifested that miR-21 is up-regulated about six times in oocytes and about 25 times in cumulus cells during *in vitro* maturation of pig oocytes. Based on the research of Han et al. (31) and Carletti et al. (32), oocyte-secreted factors (OSFs) promoted expression of miR-21 by targeting transforming growth factor-β (TGF-β) signaling, while miR-21 quenched apoptosis of cumulus and periovulatory granulosa cells by triggering the phosphatidylinositol 3-kinase (PI3K)/Akt signaling and decreasing cleaved caspase-3, respectively. The PI3K signaling pathway was described as a cell proliferation and survival regulator in the various cellular types triggered by basic fibroblast growth factor (bFGF). In addition, cleaved caspase-3 was considered a key actor of nuclear changes associated with apoptotic processes (33, 34). Furthermore, the results of Pan and Li (24) demonstrated that miR-21 enhanced porcine oocyte maturation and cumulus expansion by decreasing expression of tissue inhibitor of metalloproteinase-3 (TIMP3), as a matrix metalloproteinase inhibitor whose 3′-UTR sequence is targeted by this miRNA. Additionally, they proved a piece of evidence that the mentioned miRNA elevated levels of VERSICAN as well as the expressions of A disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1) and another gene associated with cumulus expansion, HAS2, in the time of COC maturation *in vitro* (24, 35). Indeed, the cleaved structure of VERSICAN, as a subset of aggregating chondroitin sulfate proteoglycans, by ADAMTS1 has a crucial role in the success of the matrix remodeling of cumulus cells (36, 37). Taken together, it seems that miR-21 can be involved in the normal activities of the ovary by affecting oocyte maturation, cumulus expansion, blastocyst and embryo development, and granulosa cell viability.

Based on growing evidence, miRNAs possess a striking role in ovarian activities. In this line, Timoneda et al. (38) explored expression levels of the porcine microRNAs by the RT-qPCR method and approved that miR-25, Let-7a, and miR-106a were expressed in ovarian tissues. McBride et al. (39) assessed the expression levels of some miRNAs at different phases of follicle development, comprising small follicles, medium follicles, pre-ovulatory follicles, early and late corpora lutea, and corpus albicans. Overall, let-7a, let-7b, miR-21, and miR-125b were the most frequently expressed miRNAs at different development phases. miR-31, miR-145, and miR-199a-3p showed a significant reduction in the follicular-luteal transition and a remarkable elevation at the follicular phase. In contrast, miR-21, miR-142-3p, and miR-503 represented a considerable increase in luteal tissues and they were normally expressed at lower levels during the follicular phases.

Different factors have function in follicle development, like TGF-β superfamily members, Smads, and activin receptor-like kinases (ALKs). Additionally, miRs can affect these agents (40-42). In this direction, it was stated that miR-224 potentiated granulosa cell proliferation by targeting Smad4, an important regulator related to follicular growth of the ovary (43). Moreover, a number of miRNAs (e.g., miR17-92 cluster and miR-183-96-182 cluster) have shown their roles in granulosa cell proliferation and differentiation as well as cell cycle transition by other mechanisms, including influencing BMPR2 and PTEN genes and FOXO1 transcription factor (44, 45). During folliculogenesis, above 99% of follicles experienced atresia, and activities of miRs in orchestrating follicle development and atresia have been illustrated (46, 47).

P-miR-1281, has-miR-936, hsa-miR-26b, hsa-miR-10b, mmu-miR-1224, P-miR-466 g-b, hsa-miR-574-5p, P-miR-1275, R-miR-26b, hsa-miR-1275, hsa-miR-149, and has-miR-99a are among overexpressed miRNAs during this degenerative process, whilst expression of has-let-7i, R-let-7a, hsa-miR-92b, P-miR-923, has-miR-92a, has-miR-1979, hsa-miR-1308, R-miR-739, hsa-miR-1826, ssc-miR-184, and P-miR-1826 were reduced during this occurrence (48-50). Folliculogenesis, as a highly dynamic occurrence, is linked with changes in circulating levels of ovarian hormones, and interestingly, the relationships between microRNAs and these hormones have also been scrutinized (51). As an example, Sirotkin et al. (52) indicated that 36 miRNAs (e.g., let-7b, let-7c, miR-17-3p, miR-15a, miR-92, miR-96, miR-108, miR-134, miR-133b, miR-146, and miR-135) suppressed progesterone secretion. In contrary, 16 miRNAs (i.e., miR-16, miR-18, miR-24, miR-25, miR-32, miR-103, miR-122, miR-125a, miR-143, miR-145, miR-147, miR-150, miR-152, miR-153, miR-182, and miR-191) enhanced release of progesterone hormone in granulosa cells. Regarding testosterone hormone, this study determined that let-7a, let-7b, let-7c, miR-17-3p, miR-16, miR-24, miR-26a, miR-25, miR-122, miR-108 repressed release of the aforementioned hormone. In addition, it was expressed that miR-378 influenced synthesis of estradiol hormone by binding 3′-

UTR of the aromatase coding sequence (53). miRNAs can also function in ovarian activities indirectly. Hasuwa and co-workers studied effects of miR-200b and miR-429 on infertility and anovulation in female mice, and finally they found that miR-200b and miR-429 abrogated zinc-finger E-box binding homeobox 1 (ZEB1) expression in the pituitary gland, whereby expression of these miRNAs were remarkably high. Plus, miR-200b and miR-429 inhibition suppressed luteinizing hormone (LH) biosynthesis, revealing that these miRNAs facilitated ovulation indirectly by affecting the hypothalamus-pituitary-ovarian axis (54). In summing up, it looks like the normal action of the ovary is regulated or mediated by the certain miRNAs.

It was addressed that miR-21 expression was elevated simultaneously with an increase in LH level after, during, and before ovulation (55). Interestingly, the increment LH level, causing disruption of ovarian folliculogenesis and change in production of steroid hormones, was commonly observed in PCOS women (56). miR-21 was also upregulated in granulosa cells, blood, and follicular fluid of patients with PCOS (57). Ovarian follicular fluid was produced from theca and granulosa cells. It is known as a necessary microenvironment for oocyte development and maturation (58). According to the work performed by Yu et al. (21), miR-21 can be involved in the inflammatory events of PCOS through regulation of cell proliferation and apoptosis of granulosa cells by affecting toll-like receptor 8 (TLR8). They found that expression levels of TLR8 and miR-21 were remarkably elevated in granulosa cells of PCOS cases, in comparison with the normal granulosa cells. In this regard, miR-21 elevated mRNA translation of TLR8 and consequently enhanced release of inflammatory agents, including interleukin-12 (IL-12), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). TLR8, as a subset of the family of TLRs, is dominantly expressed in myeloid dendritic cells, macrophages, and monocytes. It has a substantial role in inflammatory reactions (59, 60). Hilker et al. (26), in another scientific endeavor, inspected miR-21-5p function in porcine granulosa cells by RT-qPCR technique. They determined that this non-coding RNA was dramatically higher in granulosa cells obtained from large antral follicles, rather than those from small antral follicles. Moreover, they revealed that miR-21-5-p curbed Wilms tumor gene (WT1) expression (Fig.1), expressed by follicular cells, via binding to the 3′-UTR sequence of WT1 in granulosa cells to potentiate estradiol synthesis and aromatase expression (26, 61). Aromatase enzyme, a converter of androgens to estradiol, was significantly stimulated in human and animal cases of PCOS (62). However, Aldakheel et al. (63) and Ren et al. (64) declared that miR-21 can be an inhibitor factor for PCOS progression through suppression of proliferation of granulosa cells by affecting SNHG7, a subclass of SMAD protein family involved in cell apoptosis and proliferation adjustment. Additionally, in this new investigation, there was a reduction in the expression levels of miR-21 in ovarian tissue samples of PCOS subjects in comparison with the normal ovarian tissue samples (63). According to the majority of documents, miR-21 dysregulation may be linked with the pathogenic occurrences of PCOS; however, more *in vivo* and *in vitro* works are offered to be carried out to express its exact role in these conditions.

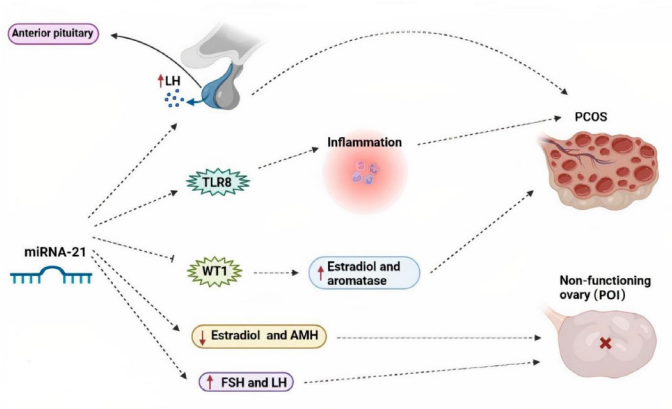

Fig.1: The possible pathogenic effects of miR-21 in the onset or progression of infertility-related disorders, like PCOS and POI. PCOS; Polycystic ovarian syndrome, POI; Premature ovarian insufficiency, FSH; Follicle-stimulating hormone, LH; Luteinizing hormone, AMH; Anti-müllerian hormone, WT1; Wilms tumor gene, and TLR8; Toll-like receptor 8.

miR-21 dysregulation has been investigated in a few studies. In the study performed by Li et al. (65) on POI patients and animal model of the disease, caused by zona pellucida glycoprotein 3 (ZP3) antigen immunization, decreased expression of this single-stranded RNA was approved. Moreover, miR-21 had direct relationship with ovarian volume, uterus size, E2 and anti-Müllerian hormone (AMH). It also had an inverse relationship with LH and FSH levels, and a number of immune indices, including anti-endometrial antibody (EMAb), anti-ovarian tissue antibody (AOAb), anti-cardiolipin antibody (ACL), anti-double-stranded DNA antibody (ds-DNA), anti-adrenal cortical antibody (ACA), anti-nuclear antibody (ANA), immunoglobulin E (IgE), IgM, IgA, IgG, serum levels of complement 3 (C3) and complement 4 (C4). Finally, these results showed that this miRNA may be related to autoimmune POI pathogenesis. In another scientific project, effects of rat mesenchymal stem cells (MSCs) transfected by miR-21 on animal models of POI were studied. In this study, POI model was induced by intraperitoneal injection of a chemotherapeutic agent, cyclophosphamide. Unlike the previous study, this research team approved that transplanting the MSCs over-expressing miR-21 had reparative impacts on POI rats by upregulating expression of the miRNA in the ovary and subsequently reducing apoptosis of granulosa cells, elevating the follicle number, ovarian weight, and E2 levels, in addition to decreasing FSH levels (66). According to these studies, it is proposed that miR-21, based on its presence in different media, may play a positive or negative role against POI; however, more and large investigations are needed to demonstrate this theory.

Dysregulative and pathogenic roles of some miRNAs in ovarian function-associated infertility have been

documented. In a scientific effort, using TaqMan miRNA and Genome-wide deep sequencing assays, Sang et al. evaluated miRNA expression in human follicular fluid of PCOS cases. They observed that expression of miR-132 and miR-320 was reduced in the patient compared to the normal group. Other findings of this study implicated that miR-24, miR-132, miR-222, miR-320, and miR-520c-3p affected estradiol secretion, while miR-24, miR-483-5p, and miR-193b influenced progesterone release in PCOS patients (67). A preliminary study explored miRNA expression profile by TaqMan RT-qPCR method on 36 PCOS women and 16 normal subjects. It was shown that circulating levels of miR-26a-5p, miR-23a-3p, miR-21-5p were upregulated, whereas miR-222-3p, miR-19b-3p, miR-376a-3p, and miR-103a-3p were downregulated in PCOS subjects rather than normal individuals. Furthermore, miR-376a-3p, miR-21-5p, and miR-103a-3p were associated with total testosterone levels (68). The actions of some microRNAs have remained a challenging issue in ovarian disorders. For example, miR-483-5p is one of the challenging miRNAs in granulosa cells (69). A scientific work revealed upregulation of miR-483-5p in the granulosa cells obtained from PCOS cases, which may reflect its role in ectopic regulation of proliferation and apoptosis in these cells by targeting Notch-3 (Notch homolog 3) gene (70). On the other hand, the other authors observed reduced expression of this miRNA in the granulosa cells of PCOS subjects which may affect insulin-like growth factor-1 (IGF-1) and subsequently potentate granulosa cell proliferation (71, 72). Concerning POI, studies have been performed by notice to both ovarian tissue and plasma samples to determine miRNAs involved in POI development. Dang and colleagues inspected expression of plasma miRNAs based on the data of the microarray platform and RT-qPCR method between women with or without POI. Eventually, this study accentuated that miR-22-3p was a protective agent for this condition and it was negatively correlated with serum levels of the FSH hormone (73). Another research team identified 20 downregulated and 63 upregulated miRNAs in the samples of ovarian tissue from the rat POI model, caused by 4-vinylcyclohexene diepoxide (VCD), than the normal group. miR-144 and miR-29a, regulators of prostaglandin secretion via affecting phospholipase A2 group IVA (PLA2G4A), were downregulated in POI tissue samples; however, several miRNAs were upregulated, such as miR-672, miR-151, miR-190, and miR-27b.a, which play roles in apoptotic process (74). Another report also demonstrated that miR-23a was upregulated in the plasma samples of POI subjects, which in turn elevated apoptosis and diminished caspase-3 and X-linked inhibitor of apoptosis protein (XIAP) levels in human granulosa cells (75). These findings revealed that abnormal function of the ovary in PCOS and POI was along with the dysregulation of many miRNAs, influencing the ovarian structure and hormone secretion (Table 1).

Conclusion

It seems that miR-21 has a substantial role in processes leading to fertility, such as oocyte maturation, cumulus expansion, inhibition of cumulus and periovulatory granulosa cell apoptosis, transition from germinal vesicle to oocytes (arrested in the MII stage), blastocyst and embryo developments. However, their dysregulation may be involved in infertility-related conditions, like PCOS and POI, by different mechanisms. For example, miR-21 dysregulation by influencing TLR8, promoting secretion of inflammatory factors (e.g., IL-12, TNF-α, and IFN-γ), and inhibiting WT1 expression had a pathogenic role in PCOS. On the other hand, miR-21 malfunction exerted its negative role in POI by decreasing E2 and AMH and increasing LH and FSH levels. Thus, dysregulation of miR-21 can be associated with the pathogenic events of PCOS and POI. Despite these, multiple in vivo and in vitro investigations are required to determine pathogenic role(s) of miR-21 in these problems.

Table 1: Function of miR-21 in ovarian function-related disorders, including PCOS and POI

Ovarian function-related diseases	Expression	Targets	Mechanisms/influences/associations	Model (human/animal)	References
PCOS	Upregulation	TLR8	Potentiating IL-12, TNF-α, and IFN-γ levels and granulosa cell proliferation	Human	(21)
PCOS	Downregulation	SNHG7	Suppressing ovarian granulosa cell proliferation	Human	(63)
PCOS	Downregulation	-	Attenuating body weight and ameliorating energy expenditure	Animal	(76)
PCOS	Upregulation	-	Decreasing insulin resistance	Animal	(77)
POI	Downregulation		Positive associations with E2, AMH, ovarian volume and negative associations with LH, FSH and the number of positive immune parameters (AOAb, EMAb, ACL, ANA, dsDNA, ACA, IgG, IgA, IgM, IgE, C3, and C4)	Animal/human	(78)
POI	-	PTEN and PDCD4	Suppressing granulosa cell apoptosis	Animal	(66)

PCOS; Polycystic ovarian syndrome, POI; Premature ovarian insufficiency, IL-12; Interleukin 12, TNF-α; Tumor necrosis factor alpha, IFN-γ; Interferon gamma, E2; Estradiol, AMH; Anti-mullerian hormone, LH; Luteinizing hormone, and FSH; Follicle-stimulating hormone.

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Authors' Contributions

R.A., H.R.-Sh.; Have made substantial contributions to Conception and Design, Acquisition of data, Analysis and Interpretation of data. H.M., F.R.T.; Have made substantial contributions to Analysis of data. All authors have been involved in drafting the manuscript or revising it critically for important intellectual content. All authors read and approved the final manuscript.

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