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Review Article

MicroRNA-Mediated Regulation of Granulosa Cell Apoptosis: A Review

Rana R. Al-Saadi[1*], Ban Thabit Al. Ani[1], Khalid S. A. Alazzawi[2]

[1] Department of Applied Embryology, High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq

[2] High Institute for Forensic Sciences, Al-Nahrain University, Baghdad, Iraq

Abstract

MicroRNAs (miRNAs) play a critical role in regulating granulosa cell apoptosis, a fundamental process in ovarian follicular development and atresia. This review synthesizes current evidence indicating that miRNAs coordinate granulosa cell fate through 3 primary mechanistic pathways. First, the mitochondrial pathway, where miRNAs such as miR-484, miR-15a-5p, and miR-26b modulate BCL2 family proteins and cytochrome C release. Second, cell signaling cascades, particularly through the transforming growth factor-β (TGF-β) pathway, where miR-33b, miR-142, miR-423, miR-383, and miR-320 regulate various signaling components including transforming growth factor beta receptor 1 (TGFBR1) and protecting mothers against decapentaplegic homolog (SMAD) proteins. Third, metabolic regulation, where miR-34a-5p, miR-19a-3p, and miR-19b-3p influence cellular metabolism and survival through pathways such as phosphoinositide 3-kinase-protein kinase B (PI3K-Akt) and glycolysis. Dysregulation of these miRNA-mediated processes has significant implications for reproductive disorders, particularly polycystic ovary syndrome (PCOS) and premature ovarian failure. Awareness about these complex regulatory networks not only advances the knowledge of follicular development but also indicates potential therapeutic targets for treating ovarian disorders characterized by abnormal granulosa cell apoptosis.

Keywords: MicroRNA, Granulosa cell apoptosis, Ovarian follicular development, Reproductive disorders

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***Corresponding Author:** ranaalsaadi@st.nahrainuniv.edu.iq

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Introduction

Accumulated evidence has shown the microRNAs (miRNAs) to contribute effectively in regulating granulosa cell apoptosis, a critical process in ovarian follicular development and atresia.[1] These small, noncoding RNA molecules, typically 18-24 nucleotides in length, function as post-transcriptional regulators by binding to specific sequences in the 3′-untranslated region (UTR) of target messenger RNAs, leading to either mRNA degradation or translational repression.[2] In the context of granulosa cells, which are essential for nourishing oocytes and regulating follicular development, miRNAs orchestrate complex molecular pathways that determine cell fate.[3] The process is particularly significant given that in mammals, more than 99% of ovarian follicles undergo atresia, with only a small fraction reaching ovulation.[4] Multiple miRNAs have been identified as key regulators in this process, notably including the let-7 family, miR-23-27-24 cluster, miR-183-96-182 cluster, and miR-17-92 cluster.[5-8] These miRNAs primarily function through targeting crucial genes involved in cell survival and death pathways, such as protecting

mothers against decapentaplegic homolog 4 (SMAD4), SMAD5, SMAD7, mitogen-activated protein kinase kinase kinase 1 (MAP3K1), and various components of the transforming growth factor-β (TGF-β) signaling pathway.[6, 9-11] The regulatory network is more complicated by the fact that one miRNA can target hundreds of different mRNAs, while a single mRNA can be targeted by multiple miRNAs, creating an intricate web of molecular interactions that precisely control granulosa cell fate.[12, 13]

The dysregulation of miRNA-mediated control of granulosa cell apoptosis has significant pathological implications, particularly in conditions like polycystic ovary syndrome (PCOS) and premature ovarian failure (POF).[14-17] Research has showed that in PCOS patients, several key miRNAs show altered expression patterns; for instance, miR-99a is significantly downregulated while insulin-like growth factor-1 receptor (IGF-1R) is upregulated, leading to abnormal follicular development.[18] The pathological process typically manifests through 2 distinct mechanisms: first, through direct regulation of apoptotic pathways, where miRNAs like miR-23a and miR-27a promote granulosa cell apoptosis by targeting SMAD5 through the Fas ligand-Fas (FasL-Fas)-mediated pathway; and second, through modulation of steroidogenesis, where miRNAs such as miR-378 and miR-320 affect estradiol synthesis and granulosa cell proliferation by targeting key enzymes like aromatase (CYP19A1).[6, 19-21] This complex relationship becomes particularly obvious in early folliculogenesis, where the relatively lower apoptosis rate and higher proliferation rate than normal in PCOS account for the simultaneous development of multiple follicles.[22] However, as development progresses beyond the mid-antral stage, the pathological process shifts, leading to increased granulosa cell apoptosis, progressive accumulation of follicular fluid, and eventual formation of thin-walled cysts characteristic of polycystic ovaries.[22]

The clinical manifestations of disrupted miRNA-mediated granulosa cell apoptosis regulation present significant implications for female reproductive health and fertility treatment options.[23] In clinical settings, these disruptions commonly manifest as irregular menstrual cycles, infertility, and hormone imbalances, particularly evident in conditions like PCOS where patients exhibit elevated luteinizing hormone (LH) levels (approximately 11.52 IU/L compared to 6.71 IU/L in healthy individuals) and significantly higher anti-Müllerian hormone (AMH) levels (12.22 ng/mL versus 4.57 ng/mL in controls).[18] Research has revealed that specific miRNA expression profiles could serve as probable diagnostic biomarkers; for instance, differential expression of miR-146a, miR-182-5p, miR-509-3p, and miR-149-5p between mural granulosa cells and cumulus cells has diagnostic value.[24] These findings have opened new therapeutic possibilities, particularly in the context of assisted reproductive technologies.[25]

miRNAs in Granulosa Cell Apoptosis

The miRNAs, as extensively elucidated in the literature, contribute to the granulosa cell apoptosis via various mechanisms (Table 1). The pathways and mechanisms could be summarized as follows:

Table 1. Summary of Key Studies on miRNA-Mediated Regulation of Granulosa Cell Function and Apoptosis

miRNA	Study Type	Method	Result	Conclusion	Reference
Mitochondrial pathway					
miR-484	Clinical and experimental	• Analysis of granulosa cells from follicular fluid (n = 114 women) • Transfection experiments • Luciferase assays • Western blot • RT-PCR • Flow cytometry	• miR-484 was highly expressed in GCs from DOR patients • miR-484 repressed GC proliferation and induced apoptosis • miR-484 directly targeted YAP1 • miR-484 induced mitochondrial dysfunction	miR-484 contributes to DOR by regulating granulosa cell function via YAP1-mediated mitochondrial function and apoptosis	Li et,[26] 2022
miR-15a-5p	Clinical and experimental	• Analysis of follicular fluid from POR and non-POR patients (n = 45) • miRNA sequencing • RT-PCR • Western blot • Cell culture experiments	• miR-15a-5p significantly elevated in young POR group • Repressed granulosa cell proliferation through PI3K-AKT-mTOR pathway • Promoted apoptosis via BCL2 and BAD	miR-15a-5p levels correlate with poor ovarian response and may serve as a potential biomarker	Zhang et al,[27] 2017
miR-26b	Experimental	• miRNA profiling • Cell culture • Flow cytometry • DNA break analysis	• miR-26b was upregulated during atresia • miR-26b increased DNA breaks by targeting ATM	miR-26b promotes granulosa cell apoptosis through ATM targeting during follicular atresia	Lin et al,[31] 2012
miR-26b	Experimental	• Cell culture • Luciferase assays • Western blot • Flow cytometry	miR-26b targeted SMAD4 and promoted granulosa cell apoptosis	miR-26b functions as a proapoptotic factor by targeting SMAD4 in porcine follicular granulosa cells	Liu et al,[10] 2014
Cell signaling					
miR-33b	Clinical and experimental	• Granulosa cell collection from PCOS patients • RT-PCR • Luciferase reporter assays • Western blot • Cell proliferation assays	• miR-33b significantly upregulated in PCOS patients • Directly targeted TGFBR1 • Suppressed TGF-β signaling pathway • Promoted cell proliferation and reduces apoptosis	miR-33b contributes to PCOS pathogenesis through altered TGF-β signaling in granulosa cells	Li et al,[15] 2015

Table 1. Summary of Key Studies on miRNA-Mediated Regulation of Granulosa Cell Function and Apoptosis (Continued)

miRNA	Study Type	Method	Result	Conclusion	Reference
miR-142	Clinical and experimental	• Granulosa cell collection from PCOS patients • RT-PCR • Luciferase reporter assays • Western blot • Cell cycle analysis	• miR-142 significantly upregulated in PCOS patients • Directly targeted TGFBR1 • Inhibited TGF-β signaling via reduced SMAD2/3 phosphorylation • Altered cell cycle regulation	miR-142 dysregulation contributes to PCOS through disrupted TGF-β signaling and cell cycle control	Li et al,[15] 2015
miR-383	Experimental	• Granulosa cell culture • Transfection experiments • Target validation • Hormone measurements	• miR-320 regulated by miR-383 • Affected granulosa cell function • Targeted E2F1 and SF-1	miR-320 regulates granulosa cell functions through targeting specific transcription factors	Yin et al,[19] 2014
miR-383	Experimental	• Granulosa cell culture • Transfection experiments • Luciferase assays • Western blot • RT-PCR • ChIP assays	• miR-383 promoted estradiol release • Directly targeted RBMS1 • Transcriptionally regulated by SF-1 • Did not affect cell proliferation or apoptosis	miR-383 regulates steroidogenesis by targeting RBMS1, mediated by SF-1 transcriptional activation	Yin et al,[28] 2012
miR-383	Experimental	MicroRNA array assay of healthy, early atretic and progressively atretic follicles in porcine ovary	miR-383 could not be accurately measured due to low abundance in most follicle samples analyzed	No definitive conclusions could be drawn about miR-383's role due to its low expression levels	Schauer et al,[29] 2013
miR-423	Experimental	• RNA sequencing • Cell culture • Flow cytometry • Luciferase assays	• miR-423 was downregulated during atresia • it suppressed early apoptosis by targeting SMAD7	miR-423 inhibits follicular atresia initiation and early granulosa cell apoptosis	Li et al,[32] 2023

Table 1. Summary of Key Studies on miRNA-Mediated Regulation of Granulosa Cell Function and Apoptosis (Continued)

miRNA	Study Type	Method	Result	Conclusion	Reference
miR-423	Clinical and experimental (in vitro)	• Follicular fluid collection from PCOS patients and controls • Real-time PCR for miRNA quantification • Dual luciferase assay • Western blot	• miR-423 expression was downregulated in PCOS patients • miR-423 directly repressed SMAD7 expression • Affected granulosa cell apoptosis pathway	miR-423 regulates granulosa cell function through targeting SMAD7 and may be involved in PCOS pathogenesis	Li et al,[15] 2019
miR-144	Experimental (in vitro and in vivo)	• Mouse granulosa cell culture • Luciferase reporter assays • Western blot • qRT-PCR • Flow cytometry	• miR-144 targeted E2F1 and SF-1 • Regulated granulosa cell proliferation and steroidogenesis • Affected PGE2 production	miR-144 regulates granulosa cell functions by targeting E2F1/SF-1 and modulating the PGE2 pathway	Zhou et al,[33] 2017
miR-144	Clinical and experimental	• RNA sequencing • Cell proliferation assays • Luciferase reporter assays • Western blot	miR-144 regulated granulosa cell function through multiple pathways (cell proliferation and steroidogenesis pathways)	miR-144 plays a role in granulosa cell regulation	Chen et al,[34] 2023
miR-320	Clinical and experimental (in vitro)	• Collection of human follicular fluid • Real-time PCR • Embryo quality assessment • Cell culture experiments	• miR-320 levels correlated with embryo quality • Affected granulosa cell function • Regulated steroidogenesis	miR-320 could serve as a biomarker for embryo quality and impacts follicular development	Yin et al,[19] 2014
miR-320	Clinical and experimental (in vitro)	• Follicular fluid analysis • miRNA expression profiling • Cell proliferation assays • Hormone analysis	• miR-320 expression affected by melatonin levels • Impacted embryo development • Regulated cell proliferation	miR-320 participates in melatonin-mediated regulation of embryo development	Khan et al,[35] 2021

Table 1. Summary of Key Studies on miRNA-Mediated Regulation of Granulosa Cell Function and Apoptosis (Continued)

miRNA	Study Type	Method	Result	Conclusion	Reference
miR-873	Experimental	• Cell culture experiments • RT-PCR • Western blot • Luciferase assays	miR-873 regulated granulosa cell function and survival through specific signaling pathways	miR-873 serves as a regulatory factor in granulosa cell function and follicular development	Sontakke et al,[36] 2014
Metabolic regulation					
miR-34a-5p	Clinical and experimental	• Human KGN cells analysis • Clinical sample analysis • Metabolic pathway studies • Western blot • RT-PCR	• Directly targeted LDHA, impaired glycolysis through upregulation of proapoptotic factors • Reduced energy availability in granulosa cells through upregulation of proapoptotic factors • Promoted apoptosis through upregulation of proapoptotic factors	miR-34a-5p orchestrates a complex regulatory network linking metabolic dysfunction to cell death in granulosa cells	Cui et al,[37] 2024
miR-19a-3p	Clinical and experimental	• Follicular fluid analysis • miRNA profiling • Cell culture experiments • RT-PCR • Pathway analysis	• miR-19a-3p differentially expressed in PCOS patients • Downregulated upon copper exposure in granulosa cells • Targeted genes in PI3K-Akt and FOXO pathways • Altered expression precedes apoptosis	miR-19a-3p works as a molecular mediator in stress response pathways affecting granulosa cell survival	Chen et al,[34] 2023; Cui et al,[54] 2021
miR-19b-3p	Clinical and experimental	• Exosomal miRNA profiling • KEGG pathway analysis • RT-PCR • Functional assays	• Significantly upregulated in PCOS patients • Associated with metabolic pathway regulation • Participated in PI3K-Akt and MAPK signaling • Influenced granulosa cell apoptosis	miR-19b-3p functions as a regulatory molecule in metabolic pathways affecting granulosa cell fate	Xie et al,[55] 2016; Ye et al,[56] 2021

Table 1. Summary of Key Studies on miRNA-Mediated Regulation of Granulosa Cell Function and Apoptosis (Continued)

miRNA	Study Type	Method	Result	Conclusion	Reference
miR-99a	Clinical and experimental	• Analysis of granulosa cells from PCOS patients • Cell culture experiments • Target validation studies	• miR-99a was significantly downregulated in PCOS patients • IGF-1R was upregulated • Abnormal follicular development	miR-99a dysregulation contributes to PCOS pathogenesis through altered IGF-1R signaling	Geng et al,[18] 2019
miR-27a-3p	Experimental	• MicroRNA profiling • Cell culture studies • Luciferase assays	• miR-27a-3p was upregulated during follicular atresia • It promoted granulosa cell apoptosis by targeting ATM gene	miR-27a-3p functions as a proapoptotic factor in porcine granulosa cells through ATM regulation	Tao et al,[30] 2023

Abbreviations: ATM, ataxia telangiectasia mutated; BAD, BCL2-associated agonist of cell death; ChIP, chromatin immunoprecipitation; DOR, diminished ovarian reserve; E2F1, E2F transcription factor 1; GCs, granulosa cells; IGF-1R, insulin-like growth factor-1 receptor; LDHA, lactate dehydrogenase A; PGE2, prostaglandin E2; PCOS, polycystic ovary syndrome; PI3K-Akt, phosphoinositide 3-kinase-protein kinase B, POR, poor ovarian response; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RBMS1, RNA binding motif single stranded interacting protein 1; RT-PCR, reverse transcription-polymerase chain reaction; SF-1, steroidogenic factor 1; SMAD, mothers against decapentaplegic homolog; TGF-β, transforming growth factor-β; TGFBR1, transforming growth factor beta receptor 1; YAP1, Yes-associated protein 1.

1) Mitochondrial Pathways

miR-484

Two significant studies have particularly emphasized the importance of miR-484 in this process through distinct but related mechanisms. Wang et al[39] demonstrated that miR-484 mediates oxidative stress-induced ovarian dysfunction by targeting SESN2, leading to increased mitochondrial reactive oxygen species (ROS) production, compromised mitochondrial membrane potential, and reduced ATP levels in granulosa cells. The study showed that miR-484 overexpression disrupted mitochondrial dynamics and activated the mitochondrial apoptotic pathway, evidenced by increased cytochrome C release and elevated expression of proapoptotic proteins. In addition, Li et al[26] revealed another mechanism through which miR-484 impacts mitochondrial function by targeting Yes-associated protein 1 (*YAP1*), showing that increased miR-484 expression leads to mitochondrial dysfunction characterized by fragmented mitochondrial networks and depolarized mitochondrial membrane potential. Both studies demonstrated that rescuing the respective targets (SESN2 or YAP1) could restore mitochondrial function and prevent apoptosis, suggesting that miR-484 serves as a central regulator of mitochondrial-mediated granulosa cell (GC) apoptosis through multiple pathways. These findings have recognized miR-484 as a key mediator of mitochondrial-dependent granulosa cell death and suggest that targeting this miRNA or its downstream effectors could provide therapeutic opportunities for treating ovarian disorders characterized by excessive GC apoptosis.

miR-15a-5p

Another regulator is miR-15a-5p, which has been reported in several studies. Of particular significance, Zhang et al[27] demonstrated through miRNA sequencing and functional studies that elevated miR-15a-5p levels in poor ovarian response patients significantly impact the intrinsic apoptotic pathway. They showed that high levels of miR-15a-5p repressed granulosa cell proliferation and promoted apoptosis through direct regulation of BCL2 and BCL2-associated agonist of cell death (BAD), which are key regulators of mitochondrial-mediated apoptosis. Using KGN cell line experiments, they demonstrated that miR-15a-5p mimic transfection significantly decreased BCL2 expression while increasing proapoptotic markers. This finding was further supported by Wang et al[39] who, although studying preeclampsia, revealed that miR-15a-5p regulates the expression of apoptosis-related proteins including cleaved-caspase-3, BCL2-associated X protein (BAX), and BCL2, suggesting a conserved role in mitochondrial apoptotic signaling across different reproductive pathologies. Especially, both studies identified the PI3K/AKT pathway as a fundamental mediator of these effects, with Zhang et al,[27] showing that miR-15a-5p inhibits this survival pathway in granulosa cells, leading to increased mitochondrial-dependent apoptosis.[27] This mechanism was further elucidated by Naji et al[40] in their PCOS study, where they observed differential expression of miR-15a-5p between granulosa cells and follicular fluid, suggesting a complex regulatory network in the control of cell survival and death pathways. Collectively, these findings establish miR-15a-5p as a critical regulator of the mitochondrial apoptotic pathway in granulosa cells, primarily through its modulation of BCL2 family proteins and the PI3K/AKT signaling axis, contributing to our understanding of how microRNA dysregulation may lead to reproductive pathologies through altered granulosa cell survival.

miR-26

Numerous studies examining the molecular mechanisms of granulosa cell apoptosis have identified miR-26b as a significant regulator of cell death pathways. Liu et al[41] presented compelling evidence that miR-26b functions as a proapoptotic factor in porcine granulosa cells through mechanisms involving the mitochondrial death pathway. Using flow cytometry and molecular analyses, they demonstrated that miR-26b overexpression significantly altered the balance of key mitochondrial pathway proteins, decreasing antiapoptotic Bcl-2 levels while promoting expression of the proapoptotic protein BAX. Through detailed mechanistic studies, they revealed a novel HAS2-HA-CD44-Caspase-3 pathway through which miR-26b mediates its apoptotic effects, ultimately leading to activation of the intrinsic mitochondrial apoptotic cascade. Importantly, inhibition of miR-26b protected against granulosa cell apoptosis by preserving the expression of antiapoptotic factors, with a demonstrated increase in BCL2 and decrease in caspase-3 activation. Other studies have shown that miR-26 family members can promote granulosa cell apoptosis through different mechanisms; such as targeting *DHCR24* or *Ezh2*.[41, 42] These findings have shown miR-26b as a regulator of the intrinsic apoptotic pathway in granulosa cells, although further research is still needed to fully elucidate its interactions with other components of the mitochondrial death machinery.

Studies have demonstrated that miR-26b plays a significant role in promoting granulosa cell apoptosis through mitochondrial-dependent pathways. Liu et al[41] offered compelling evidence that miR-26b functions as a proapoptotic factor by directly targeting the *HAS2* gene, leading to downstream activation of the mitochondrial apoptotic pathway.

Through flow cytometry and molecular analyses, they demonstrated that miR-26b overexpression significantly decreased antiapoptotic BCL2 levels while promoting expression of the proapoptotic protein BAX. The study revealed a novel HAS2-HA-CD44-Caspase-3 pathway through which miR-26b mediates its apoptotic effects, ultimately leading to mitochondrial membrane permeabilization and cytochrome C release. This mechanism was further supported by Zhang et al[43] who showed that miR-26b's proapoptotic effects involve targeting *DHCR24*, which result in similar alterations in the BCL2/BAX ratio and subsequent activation of the intrinsic mitochondrial apoptotic pathway. Obviously, both studies demonstrated that inhibition of miR-26b protected against granulosa cell apoptosis by preserving mitochondrial integrity through maintenance of antiapoptotic protein expression. The importance of miR-26b in mitochondrial-mediated apoptosis was additionally corroborated by Huo et al[42] who showed that miR-26 family members (particularly miR-26a) regulate the expression of key apoptotic mediators involved in the mitochondrial death pathway. Together, these findings have shown miR-26b as a central regulator of the intrinsic apoptotic pathway in granulosa cells, suggesting its potential as a therapeutic target for disorders involving abnormal follicular atresia or granulosa cell death.

2) Cell Signaling

miR-33b

Research has revealed significant contributions of miR-33b to granulosa cell signaling pathways and survival mechanisms, particularly in the context of PCOS. In 2019, Li et al[15] reported that miR-33b is significantly upregulated (P = .032) in granulosa cells of PCOS patients and directly targets the transforming growth factor beta receptor 1 *(TGFBR1)* through specific binding to its 3′ UTR region. This targeting leads to suppression of the TGFB signaling pathway, which normally regulates cell proliferation and apoptosis. When combined with other miRNAs dysregulated, elevated miR-33b levels promote granulosa cell survival by increasing cell proliferation (P = .0098) and significantly reducing apoptotic rates (P = .027). This antiapoptotic effect is accompanied by cell cycle modifications, specifically an increase in S phase cell numbers (P = .0036), indicating enhanced cell division. Previous research has also linked miR-33b to metabolic regulation in PCOS, with Yang et al[44] showing its involvement in glucose transport through glucose transporter type 4 (GLUT4) regulation, suggesting a multifaceted role in PCOS pathogenesis. The dysregulation of miR-33b thus appears to contribute to the abnormal follicular development characteristic of PCOS through both direct effects on granulosa cell survival and broader metabolic impacts.

miR-142

The miR-142 is also reported in modulating granulosa cell apoptosis through complex interactions with the TGF-β signaling pathway. In a study by Li et al[15] they found that miR-142 is significantly upregulated in granulosa cells of PCOS patients (P = .021) and directly targets *TGFBR1* through binding sites in its 3′ UTR, as confirmed through dual luciferase reporter assays. This targeting relationship was shown to have functional consequences, as elevated miR-142 levels led to decreased TGFBR1 protein expression and subsequent inhibition of TGF-β signaling, evidenced by reduced phosphorylation of downstream effectors SMAD2 and SMAD3. The functional significance of this regulatory axis was demonstrated through cell-based assays, where miR-142 overexpression promoted granulosa cell proliferation and suppressed apoptosis, particularly by increasing

the proportion of cells in S-phase. These findings aligned with previous work showing miR-142's role as a TGF-β signaling repressor in other cell types,[45-47] but uniquely established its importance in ovarian function. Mechanistically, the disruption of TGF-β signaling by elevated miR-142 was found to alter the expression of key cell cycle regulators, with significant downregulation of *CDKN1A* and *CDKN2B* and upregulation of c-*MYC*, creating a molecular environment that favors cell survival over apoptosis. This pathway appears particularly relevant to PCOS pathogenesis, as patient samples consistently showed this pattern of elevated miR-142, reduced TGFBR1, and altered downstream signaling, suggesting that targeting this axis could have therapeutic potential in treating PCOS-related ovarian dysfunction.

miR-423

Several recent studies have supported evidence for miR-423's critical role in regulating granulosa cell function and apoptosis through complex signaling pathways. One study showed that miR-423 acts as a key inhibitor of granulosa cell apoptosis, particularly in the early stages, by directly targeting and repressing SMAD7 expression through interaction with its 3'UTR region.[32] This was further supported by Xu et al[48] who showed that miR-423 expression was significantly downregulated in granulosa cells from PCOS patients, leading to dysregulated cell proliferation and survival. The mechanistic roles were expanded by Xie et al[49] who revealed that miR-423 modulates granulosa cell function through the CSF1 pathway, affecting both cell survival and estradiol synthesis. Collectively, these studies have established a regulatory network where miR-423 functions as a central mediator in the TGF-β signaling pathway by targeting multiple components; most notably SMAD7, which has been consistently identified across studies as a direct target. When miR-423 levels are reduced, as observed in pathological conditions like PCOS, the resulting increase in SMAD7 expression leads to enhanced granulosa cell apoptosis and disrupted follicular development. This has been conclusively demonstrated through various experimental approaches, including luciferase reporter assays, flow cytometry analyses, and functional studies using miRNA mimics and inhibitors, which showed that restoring miR-423 levels could effectively suppress granulosa cell apoptosis and normalize TGF-β signaling. These findings not only illuminate the molecular mechanisms underlying granulosa cell regulation, but also suggest potential therapeutic strategies targeting the miR-423/SMAD7/TGF-β axis for treating ovarian disorders characterized by abnormal granulosa cell apoptosis.

miR-383

Several studies have provided compelling evidence for the critical role of miR-383 in regulating granulosa cell apoptosis through complex signaling pathways, particularly in the context of polycystic ovary syndrome (PCOS). Remarkably, Yin et al[19] demonstrated that miR-383 expression was significantly downregulated in TGF-β1-treated mouse granulosa cells, where it targeted E2F transcription factor 1 (E2F1) and steroidogenic factor 1 (SF-1) to regulate cell proliferation and steroidogenesis. Building on this foundation, Li et al[50] revealed a novel regulatory axis involving miR-383-5p and cold-inducible RNA binding protein (CIRP) in human granulosa cells. Their research showed that miR-383-5p expression was markedly decreased in PCOS patients' granulosa cells, exhibiting negative correlations with clinical parameters including body mass index (BMI), luteinizing hormone (LH) levels, and testosterone concentrations. Through sophisticated molecular analyses including

luciferase reporter assays and Western blotting, they established that miR-383-5p directly targets CIRP, which subsequently modulates the PI3K/AKT signaling pathway. The researchers demonstrated that overexpression of miR-383-5p enhanced granulosa cell apoptosis by suppressing CIRP expression, leading to decreased phosphorylation of PI3K and AKT, and increased expression of proapoptotic proteins including BAX and cleaved caspase-3. This mechanistic understanding was further validated through rescue experiments showing that CIRP overexpression could partially reverse the proapoptotic effects of miR-383-5p.[50] Collectively, these studies establish miR-383 as a master regulator of granulosa cell fate through its intricate modulation of multiple signaling pathways, including both the classical PI3K/AKT pathway and steroidogenic regulatory networks, suggesting its potential as both a diagnostic marker and therapeutic target in PCOS treatment.

miR-320

Based on the comprehensive analysis of recent studies, miR-320 has emerged as a critical regulator of granulosa cell function and survival through multiple signaling pathways. A study showed that miR-320 directly targets the runt-related transcription factor 2 (RUNX2) in cumulus granulosa cells, thereby modulating the expression of steroidogenic enzymes CYP11A1 and CYP19A1.[21] This regulation was shown to be IGF-1-dependent, as their in vitro studies revealed that IGF-1 stimulation significantly upregulated miR-320 expression in normal granulosa cells after 24 hours of treatment. Furthermore, Yin et al[19] established through luciferase reporter assays that miR-320 also targets E2F1 and SF-1, key transcription factors involved in cell cycle regulation and steroidogenesis. Their functional studies have demonstrated that miR-320 overexpression significantly increased granulosa cell apoptosis, as evidenced by enhanced cleavage of caspase-3 and poly(ADP-ribose) polymerase (PARP) proteins. More recent clinical research by Liu et al[51] corroborated these findings, and showed that altered miR-320 levels in granulosa cells correlate with cellular function and survival outcomes. Through reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of 195 patient samples, they found that higher miR-320 expression levels were associated with increased apoptotic markers and reduced cell viability. To conclude, these studies have established a complex regulatory network where miR-320 influences granulosa cell fate through multiple mechanisms: direct regulation of steroidogenic pathways via RUNX2/CYP11A1/CYP19A1 signaling, modulation of cell cycle progression through E2F1 targeting, and regulation of apoptotic pathways through caspase-dependent mechanisms. This multilayered control emphasizes miR-320's crucial role in maintaining granulosa cell homeostasis and suggests its potential as a therapeutic target for reproductive disorders characterized by aberrant granulosa cell function.

miR-144

Research on miR-144's role in granulosa cell function also showed significant impact on cell survival and steroidogenesis through complex regulatory mechanisms. Zhou et al[33] found that miR-144 directly targets transcription factors E2F1 and SF-1, which are key regulators of granulosa cell proliferation and steroidogenic activity. Their research showed that miR-144 expression is regulated by the transcription factor CP2 and influences prostaglandin E2 (PGE2) production, which is important for ovulation and luteinization. The study utilized both in vitro and in vivo approaches, including luciferase reporter assays and flow cytometry, to establish that miR-144 modulates granulosa

cell functions through multiple pathways. This finding was corroborated by additional research[34] that confirmed miR-144's regulatory effects on granulosa cell proliferation and steroidogenesis. The multi-targeted nature of miR-144 suggests its possible role as a master regulator in ovarian function, coordinating both cell cycle progression and hormone production.

3) Metabolic Regulation

miR-34a-5p

Recent studies have provided compelling evidence for miR-34a-5p's central role in metabolic regulation and granulosa cell apoptosis, particularly in the context of ovarian dysfunction. Han et al[52] demonstrated through in vitro studies using chicken follicular granulosa cells that miR-34a-5p promotes both autophagy and apoptosis by targeting lymphoid enhancer-binding factor 1 (LEF1) and modulating the Hippo-Yes-associated protein (YAP) signaling pathway. This finding was corroborated by Fabová et al[53] who utilized porcine ovarian granulosa cells to show that miR-34a-5p inhibits cell proliferation while promoting apoptosis through the regulation of key proteins including BAX and caspase-3. Most recently, Cui et al[37] provided crucial mechanistic insights through their work with human KGN cells and clinical samples, demonstrating that miR-34a-5p directly targets lactate dehydrogenase A (LDHA), leading to impaired glycolysis and reduced energy availability in granulosa cells. Their research revealed a clear metabolic pathway where elevated miR-34a-5p levels, particularly evident in PCOS patients, suppress glycolytic enzyme activity (including HK2 and PKM2) and reduce pyruvate-to-lactate conversion, ultimately promoting granulosa cell apoptosis through the upregulation of proapoptotic factors and downregulation of antiapoptotic BCL2. Together, these studies have established a comprehensive understanding of how miR-34a-5p orchestrates a complex regulatory network that links metabolic dysfunction to cell death in granulosa cells, suggesting its potential as both a diagnostic marker and therapeutic target in ovarian disorders characterized by aberrant follicular development and increased granulosa cell apoptosis.

miR-19a-3p

Some other studies have investigated miR-19a-3p and confirmed it as significant regulator in granulosa cell apoptosis and metabolic pathways, particularly under copper-induced stress conditions. One of these is the 2021 Cui et al's[54] study which proved through microRNA profiling of follicular fluid that miR-19a-3p was differentially expressed between women with and without PCOS, suggesting its potential role in ovarian dysfunction. This finding was further substantiated by Chen et al[34] who observed that copper exposure significantly downregulated miR-19a-3p expression in human luteinized granulosa cells across multiple treatment concentrations (0.5-10.0 μg/mL). Through bioinformatic analyses and pathway mapping, they revealed that miR-19a-3p targets genes involved in critical signaling cascades, including the phosphoinositide 3-kinase-protein kinase B (PI3K-Akt) and FOXO signaling pathways, which are fundamental to cell survival and apoptotic regulation. The researchers validated these findings using RT-qPCR, demonstrating that the downregulation of miR-19a-3p coincided with increased expression of apoptotic markers and activation of the caspase-dependent pathway. Notably, the altered expression of miR-19a-3p was observed to precede visible signs of cellular apoptosis, suggesting its potential role as an early molecular mediator in the stress response pathway.

This regulatory relationship indicates that miR-19a-3p may serve as a critical molecular switch in determining granulosa cell fate, potentially through its modulation of metabolic and survival pathways. Understanding this mechanism could provide valuable insights into both the pathogenesis of ovarian disorders and potential therapeutic approaches for maintaining granulosa cell health.

miR-19b-3p

Several studies have explained the probable role of miR-19b-3p in metabolic regulation pathways leading to granulosa cell apoptosis. It was hypothesized, through RNA sequencing and RT-qPCR validation, that copper exposure significantly altered miR-19b expression patterns in ovarian granulosa cells, with the miRNA being involved in the activation of the caspase-dependent apoptotic signaling pathway.[34] This finding was corroborated by Xie et al[55] who identified miR-19b-3p as one of the differentially expressed miRNAs in granulosa cells of ovarian hyperresponders, with their pathway analysis revealing its involvement in metabolic processes and cell proliferation regulation. Particularly, Ye et al[56] provided further evidence through exosomal miRNA profiling of follicular fluid, showing that miR-19b-3p was significantly upregulated (log_{FC}: 1.724336734, P = .041056) in PCOS patients and was associated with metabolic pathway regulation through KEGG pathway analysis. The study identified that miR-19b-3p participated in crucial metabolic signaling networks, including the PI3K-Akt and MAPK pathways, which are known regulators of granulosa cell survival and apoptosis. Interestingly, Nagata et al[57] found that miR-19b was highly abundant in the follicular fluid of young cows and demonstrated its importance in oocyte development, suggesting an age-dependent regulatory role. Collectively, these studies have established miR-19b-3p as a key regulatory molecule in metabolic pathways that influence granulosa cell fate, particularly through its involvement in apoptotic signaling cascades, making it a potential therapeutic target for ovarian disorders characterized by aberrant granulosa cell apoptosis.

miR-99

Reports on miR-99a have supported its vital role in regulating metabolic pathways and granulosa cell survival in the context of PCOS. Geng et al[18] conducted a comprehensive study demonstrating that miR-99a is significantly downregulated in granulosa cells of PCOS patients compared to controls. Through rigorous molecular analyses, they established that miR-99a directly targets IGF-1R, a key component of metabolic signaling pathways in ovarian cells. The researchers observed that the diminished expression of miR-99a corresponded with increased IGF-1R levels, leading to dysregulated cell proliferation and abnormal follicular development characteristic of PCOS. Functionally, their in vitro experiments showed that restoring miR-99a levels effectively reduced IGF-1R expression and normalized granulosa cell function, including proliferation rates and apoptotic patterns. This regulatory relationship was further validated through target validation studies, confirming that miR-99a's effects were specifically mediated through IGF-1R targeting. The study's findings have established miR-99a as a possible biomarker and therapeutic target for PCOS.

miR-27a-3p

Research on miR-27a-3p has revealed it plays a significant role in granulosa cell apoptosis through multiple pathways. According to Nie et al,[6] miR-27a promotes human

granulosa cell apoptosis by directly targeting SMAD5, activating the FasL-Fas signaling pathway that increases levels of Fas, FasL, cleaved caspase-8, and cleaved caspase-3. This finding has been complemented by Tao et al,[30] who demonstrated that miR-27a-3p inhibits mouse granulosa cell proliferation by targeting Vangl1 and Vangl2, which are key components in the Wnt signaling pathway. Their research showed that overexpression of miR-27a-3p significantly suppressed granulosa cell proliferation, while silencing it had the opposite effect. The myogenic differentiation (MyoD) transcription factor was identified as a regulator that binds to and activates the miR-27a-3p promoter. Together, these studies establish miR-27a-3p as an important regulator in ovarian follicle development, functioning primarily as a proapoptotic factor in granulosa cells across multiple species by targeting different genes that ultimately affect cell survival pathways.

Discussion

The involved regulation of granulosa cell apoptosis by microRNAs is a complex molecular network, that coordinates follicular development and atresia through multiple distinct but interconnected pathways. Current evidence demonstrates that these regulatory molecules primarily operate through 3 major mechanistic axes: the mitochondrial pathway, cell signaling cascades, and metabolic regulation. In the mitochondrial pathway, key players such as miR-484, miR-15a-5p, and miR-26b have been shown to modulate the expression of critical apoptotic proteins, particularly members of the BCL2 family, ultimately controlling cytochrome C release and downstream caspase activation. The significance of this regulation is particularly evident in pathological conditions, where dysregulation of these miRNAs leads to abnormal apoptotic patterns and subsequent follicular development abnormalities. For instance, studies have demonstrated that miR-484 simultaneously targets multiple proteins including SESN2 and YAP1, creating a sophisticated regulatory network that fine-tunes mitochondrial function and cellular survival.[26, 38] Also, it regulates activities of miR-15a-5p through BCL2 and BAD, and miR-26b via the HAS2-HA-CD44-Caspase-3 pathway.[27, 41] These findings together support the remarkable complexity and precision of miRNA-mediated regulation in granulosa cell apoptosis (Figure 1).

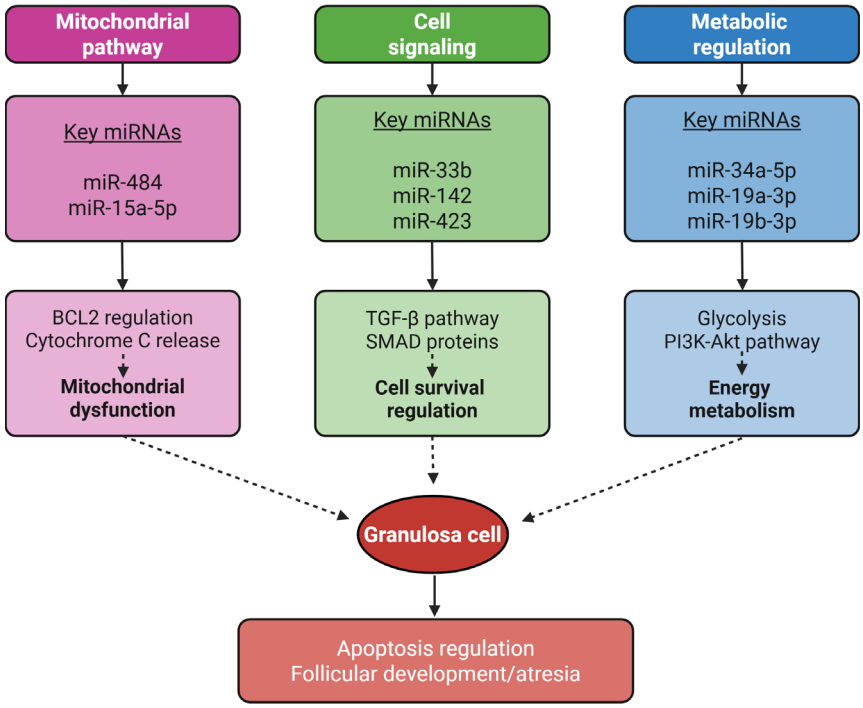
The diagram illustrates the mitochondrial pathway (involving miR-484 and miR-15a-5p, regulating BCL2 and cytochrome C), cell signaling pathway (including miR-33b, miR-142, and miR-423, modulating TGF-β and SMAD proteins), and metabolic regulation pathway (featuring miR-34a-5p, miR-19a-3p, and miR-19b-3p, affecting glycolysis and PI3K-Akt signaling). These pathways converge to control granulosa cell fate through apoptosis regulation and follicular development/atresia.

The cell signaling dimension of miRNA-mediated granulosa cell apoptosis regulation has revealed an equally sophisticated network, predominantly centered around the TGF-β signaling pathway and its associated molecules. Several key miRNAs, including miR-33b, miR-142, miR-423, miR-383, and miR-320, have been identified as crucial regulators of these signaling cascades. Of particular significance is the coordinated regulation of the TGF-β pathway through multiple miRNAs targeting different components of the signaling mechanism. For instance, both miR-33b and miR-142 directly target TGFBR1, leading to suppressed TGF-β signaling and altered cell survival outcomes in PCOS patients.[15] This regulatory complexity is further exemplified by miR-423's role in modulating SMAD7 expression and subsequent TGF-β pathway activity.[32, 48] Furthermore, miR-383 has

demonstrated the interconnected nature of these pathways through its regulation of the PI3K/AKT signaling cascade via CIRP targeting, while miR-320's simultaneous control of steroidogenic enzymes and cell cycle regulators denotes the complicated nature of these regulatory networks.[50, 51]

The metabolic dimension of miRNA-mediated granulosa cell apoptosis adds another layer of complexity to this regulatory network, particularly through the actions of miR-34a-5p, miR-19a-3p, and miR-19b-3p. Recent evidence has revealed that these miRNAs serve as critical mediators between cellular metabolism and apoptotic pathways. Most notably, miR-34a-5p has been shown to directly influence glycolytic metabolism through LDHA targeting, demonstrating how metabolic disruption can trigger apoptotic cascades in granulosa cells.[37] The importance of these metabolic regulations is further emphasized by the differential expression patterns of miR-19a-3p and miR-19b-3p observed in various pathological conditions, particularly their involvement in PI3K-Akt and FOXO signaling pathways.[34, 56] These findings collectively support the remarkable complexity and precision of miRNA-mediated regulation in granulosa cell apoptosis.

Figure 1. Three Major Pathways in microRNA-Mediated Regulation of Granulosa Cell Apoptosis



Abbreviations: SMAD, mothers against decapentaplegic homolog; TGF-β, transforming growth factor-β.

Conclusions

This review focused on the complex nature of microRNA-mediated regulation in granulosa cell apoptosis as well as its fundamental importance in ovarian function and fertility. Through the detailed examination of 3 primary regulatory mechanisms: mitochondrial pathways, cell signaling cascades, and metabolic regulation; it can be concluded that miRNAs act as necessary molecular switches controlling granulosa cell fate. The complex interaction between these pathways, coordinated by specific miRNAs targeting multiple components within each mechanism, has revealed an advanced regulatory network that maintains proper follicular development and atresia. Of particular significance is the developing evidence that dysregulation of these miRNA-mediated processes contributes significantly to reproductive disorders, especially PCOS and premature ovarian failure. However, and due to the topic's importance, future research should focus on translating these molecular targets into practical clinical applications, particularly in developing targeted treatments that can modulate specific miRNA pathways to restore normal ovarian function in pathological conditions.

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Formal Analysis: All authors
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Writing – Original Draft: All authors
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References

1. Yang L, Du X, Wang S, Lin C, Li Q, Li Q. A regulatory network controlling ovarian granulosa cell death. *Cell Death Discov*. 2023;9(1):70. doi:10.1038/s41420-023-01346-9
2. Shang R, Lee S, Senavirathne G, Lai EC. MicroRNAs in action: biogenesis, function and regulation. *Nat Rev Genet*. 2023;24(12):816-833. doi:10.1038/s41576-023-00611-y
3. Zhang J, Xu Y, Liu H, Pan Z. MicroRNAs in ovarian follicular atresia and granulosa cell apoptosis. *Reprod Biol Endocrinol*. 2019;17(1):9. doi:10.1186/s12958-018-0450-y
4. Matsuda-Minehata F, Inoue N, Goto Y, Manabe N. The regulation of ovarian granulosa cell death by pro- and anti-apoptotic molecules. *J Reprod Dev*. 2006;52(6):695-705. doi:10.1262/jrd.18069
5. Gebremedhn S, Salilew-Wondim D, Hoelker M, et al. MicroRNA-183-96-182 cluster regulates bovine granulosa cell proliferation and cell cycle transition by coordinately targeting FOXO1. *Biol Reprod*. 2016;94(6):127. doi:10.1095/biolreprod.115.137539
6. Nie M, Yu S, Peng S, Fang Y, Wang H, Yang X. miR-23a and miR-27a promote human granulosa cell apoptosis by targeting SMAD5. *Biol Reprod*. 2015;93(4):98. doi:10.1095/biolreprod.115.130690

7. Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. *Cell*. 2008;133(2): 217-222. doi:10.1016/j.cell.2008.04.001
8. Cao R, Wu W, Zhou X, et al. Let-7g induces granulosa cell apoptosis by targeting MAP3K1 in the porcine ovary. *Int J Biochem Cell Biol*. 2015;68:148-157. doi:10.1016/j.biocel.2015.08.011
9. Du X, Pan Z, Li Q, Liu H, Li Q. SMAD4 feedback regulates the canonical TGF-β signaling pathway to control granulosa cell apoptosis. *Cell Death Dis*. 2018;9(2):151. doi:10.1038/s41419-017-0205-2
10. Liu J, Du X, Zhou J, Pan Z, Liu H, Li Q. MicroRNA-26b functions as a proapoptotic factor in porcine follicular granulosa cells by targeting Sma-and Mad-related protein 4. *Biol Reprod*. 2014;91(6):146. doi:10.1095/biolreprod.114.122788
11. Yao W, Pan Z, Du X, Zhang J, Li Q. miR-181b-induced SMAD7 downregulation controls granulosa cell apoptosis through TGF-β signaling by interacting with the TGFBR1 promoter. *J Cell Physiol*. 2018;233(9): 6807-6821. doi:10.1002/jcp.26431
12. Farazi TA, Hoell JI, Morozov P, Tuschl T. MicroRNAs in Human Cancer. In: Schmitz U, Wolkenhauer O, Vera J, eds. *MicroRNA Cancer Regulation. Advances in Experimental Medicine and Biology*. Springer; 2013:1-20. doi:10.1007/978-94-007-5590-1_1
13. Gong Z, Yang J, Bai S, Wei S. MicroRNAs regulate granulosa cells apoptosis and follicular development - a review. *Asian-Australas J Anim Sci*. 2020;33(11):1714-1724. doi:10.5713/ajas.19.0707
14. Song Y, Yu G, Xiang Y, Li Y, Wan L, Tan L. Altered miR-186 and miR-135a contribute to granulosa cell dysfunction by targeting ESR2: a possible role in polycystic ovary syndrome. *Mol Cell Endocrinol*. 2019;494:110478. doi:10.1016/j.mce.2019.110478
15. Li Y, Xiang Y, Song Y, Wan L, Yu G, Tan L. Dysregulated miR-142, -33b and -423 in granulosa cells target TGFBR1 and SMAD7: a possible role in polycystic ovary syndrome. *Mol Hum Reprod*. 2019; 25(10):638-646. doi:10.1093/molehr/gaz014
16. Yang X, Zhou Y, Peng S, et al. Differentially expressed plasma microRNAs in premature ovarian failure patients and the potential regulatory function of mir-23a in granulosa cell apoptosis. *Reproduction*. 2012;144(2):235-244. doi:10.1530/REP-11-0371
17. Faisal GF, Al-Kawaz UMR, Al-Anbari LA, Hussaini HAR. Evaluation of the level of stem cell factor in follicular fluid and its effect on oocyte maturity, embryo quality and pregnancy rate. *Iraqi J Embryos Infertil Res*. 2020;9(2):65-77. doi:10.28969/IJEIR.v9.i2.r5
18. Geng Y, Sui C, Xun Y, Lai Q, Jin L. MiRNA-99a can regulate proliferation and apoptosis of human granulosa cells via targeting IGF-1R in polycystic ovary syndrome. *J Assist Reprod Genet*. 2019;36(2): 211-221. doi:10.1007/s10815-018-1335-x
19. Yin M, Wang X, Yao G, et al. Transactivation of microrn A-320 by microRNA-383 regulates granulosa cell functions by targeting E2F1 and SF-1 proteins. *J Biol Chem*. 2014;289(26):18239-18257. doi:10.1074/jbc.M113.546044
20. Xu S, Linher-Melville K, Yang BB, Wu D, Li J. Micro-RNA378 (miR-378) regulates ovarian estradiol production by targeting aromatase. *Endocrinology*. 2011;152(10):3941-3951. doi:10.1210/en.2011-1147
21. Zhang CL, Wang H, Yan CY, Gao XF, Ling XJ. Deregulation of RUNX2 by miR-320a deficiency impairs steroidogenesis in cumulus granulosa cells from polycystic ovary syndrome (PCOS) patients. *Biochem Biophys Res Commun*. 2017;482(4):1469-1476. doi:10.1016/j.bbrc.2016.12.059
22. Das M, Djahanbakhch O, Hacihanefioglu B, et al. Granulosa cell survival and proliferation are altered in polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2008;93(3):881-887. doi:10.1210/jc.2007-1650
23. Almeida CP, Ferreira MCF, Silveira CO, et al. Clinical correlation of apoptosis in human granulosa cells - a review. *Cell Biol Int*. 2018;42(10):1276-1281. doi:10.1002/cbin.11036
24. Andrei D, Nagy RA, van Montfoort A, et al. Differential miRNA expression profiles in cumulus and mural granulosa cells from human pre-ovulatory follicles. *Microrna*. 2019;8(1):61-67. doi:10.2174/2211536607666180912152618

25. McGinnis LK, Luense LJ, Christenson LK. MicroRNA in ovarian biology and disease. *Cold Spring Harb Perspect Med*. 2015;5(9):a022962. doi:10.1101/cshperspect.a022962
26. Li H, Wang X, Mu H, et al. Mir-484 contributes to diminished ovarian reserve by regulating granulosa cell function via YAP1-mediated mitochondrial function and apoptosis. *Int J Biol Sci*. 2022;18(3):1008-1021. doi:10.7150/ijbs.68028
27. Zhang K, Zhong W, Li WP, Chen ZJ, Zhang C. miR-15a-5p levels correlate with poor ovarian response in human follicular fluid. *Reproduction*. 2017;154(4):483-496. doi:10.1530/REP-17-0157
28. Yin M, Lü M, Yao G, et al. Transactivation of microRNA-383 by steroidogenic factor-1 promotes estradiol release from mouse ovarian granulosa cells by targeting RBMS1. *Mol Endocrinol*. 2012;26(7):1129-1143. doi:10.1210/me.2011-1341
29. Schauer SN, Sontakke SD, Watson ED, Esteves CL, Donadeu FX. Involvement of miRNAs in equine follicle development. *Reproduction*. 2013;146(3):273-282. doi:10.1530/REP-13-0107
30. Tao H, Yang J, Xu M, Liu Z, Liu Y, Xiong Q. MicroRNA-27a-3p targeting Vangl1 and Vangl2 inhibits cell proliferation in mouse granulosa cells. *Biochim Biophys Acta Gene Regul Mech*. 2023;1866(1):194885. doi:10.1016/j.bbagrm.2022.194885
31. Lin F, Li R, Pan ZX, et al. miR-26b promotes granulosa cell apoptosis by targeting ATM during follicular atresia in porcine ovary. *PLoS One*. 2012;7(6):e38640. doi:10.1371/journal.pone.0038640
32. Li Y, Zhang Z, Wang S, Du X, Li Q. miR-423 sponged by lncRNA NORHA inhibits granulosa cell apoptosis. *J Anim Sci Biotechnol*. 2023;14(1):154. doi:10.1186/s40104-023-00960-y
33. Zhou J, Lei B, Li H, et al. MicroRNA-144 is regulated by CP2 and decreases COX-2 expression and PGE2 production in mouse ovarian granulosa cells. *Cell Death Dis*. 2017;8(2):e2597. doi:10.1038/cddis.2017.24
34. Chen Y, Guan F, Wang P, et al. Copper exposure induces ovarian granulosa cell apoptosis by activating the caspase-dependent apoptosis signaling pathway and corresponding changes in microRNA patterns. *Ecotoxicol Environ Saf*. 2023;264:115414. doi:10.1016/j.ecoenv.2023.115414
35. Khan HL, Bhatti S, Abbas S, et al. Melatonin levels and microRNA (miRNA) relative expression profile in the follicular ambient microenvironment in patients undergoing in vitro fertilization process. *J Assist Reprod Genet*. 2021;38(2):443-459. doi:10.1007/s10815-020-02010-2
36. Sontakke SD, Mohammed BT, McNeilly AS, Donadeu FX. Characterization of microRNAs differentially expressed during bovine follicle development. *Reproduction*. 2014;148(3):271-283. doi:10.1530/REP-14-0140
37. Cui X, Lei X, Huang T, et al. Follicular fluid-derived extracellular vesicles miR-34a-5p regulates granulosa cell glycolysis in polycystic ovary syndrome by targeting LDHA. *J Ovarian Res*. 2024;17(1):223. doi:10.1186/s13048-024-01542-w
38. Wang X, Yang J, Li H, et al. miR-484 mediates oxidative stress-induced ovarian dysfunction and promotes granulosa cell apoptosis via SESN2 downregulation. *Redox Biol*. 2023;62:102684. doi:10.1016/j.redox.2023.102684
39. Wang Y, Du X, Wang J. Transfer of miR-15a-5p by placental exosomes promotes pre-eclampsia progression by regulating PI3K/AKT signaling pathway via CDK1. *Mol Immunol*. 2020;128:277-286. doi:10.1016/j.molimm.2020.10.019
40. Naji M, Nekoonam S, Aleyasin A, et al. Expression of miR-15a, miR-145, and miR-182 in granulosa-lutein cells, follicular fluid, and serum of women with polycystic ovary syndrome (PCOS). *Arch Gynecol Obstet*. 2018;297(1):221-231. doi:10.1007/s00404-017-4570-y
41. Liu J, Tu F, Yao W, et al. Conserved miR-26b enhances ovarian granulosa cell apoptosis through HAS2-HA-CD44-Caspase-3 pathway by targeting HAS2. *Sci Rep*. 2016;6:21197. doi:10.1038/srep21197
42. Huo S, Qi H, Si Y, Li C, Du W. MicroRNA 26a targets Ezh2 to regulate apoptosis in mouse ovarian granulosa cells. *Syst Biol Reprod Med*. 2021;67(3):221-229. doi:10.1080/19396368.2021.1895362

43. Zhang X, Tao Q, Shang J, et al. MiR-26a promotes apoptosis of porcine granulosa cells by targeting the 3β-hydroxysteroid-Δ24-reductase gene. *Asian-Australas J Anim Sci*. 2020;33(4):547-555. doi:10.5713/ajas.19.0173
44. Yang Y, Jiang H, Xiao L, Yang X. MicroRNA-33b-5p is overexpressed and inhibits GLUT4 by targeting HMGA2 in polycystic ovarian syndrome: an in vivo and in vitro study. *Oncol Rep*. 2018;39(6):3073-3085. doi:10.3892/or.2018.6375
45. Kim K, Yang DK, Kim S, Kang H. miR-142-3p is a regulator of the TGFβ-mediated vascular smooth muscle cell phenotype. *J Cell Biochem*. 2015;116(10):2325-2333. doi:10.1002/jcb.25183
46. Lei Z, Xu G, Wang L, et al. MiR-142-3p represses TGF-β-induced growth inhibition through repression of TGFβR1 in non-small cell lung cancer. *FASEB J*. 2014;28(6):2696-2704. doi:10.1096/fj.13-247288
47. Yu Q, Xiang L, Yin L, Liu X, Yang D, Zhou J. Loss-of-function of miR-142 by hypermethylation promotes TGF-β-mediated tumour growth and metastasis in hepatocellular carcinoma. *Cell Prolif*. 2017;50(6):e12384. doi:10.1111/cpr.12384
48. Xu X, Guan R, Gong K, Xie H, Shi L. Circ_FURIN knockdown assuages testosterone-induced human ovarian granulosa-like tumor cell disorders by sponging miR-423-5p to reduce MTM1 expression in polycystic ovary syndrome. *Reprod Biol Endocrinol*. 2022;20(1):32. doi:10.1186/s12958-022-00891-9
49. Xie S, Zhang Q, Zhao J, Hao J, Fu J, Li Y. MiR-423-5p may regulate ovarian response to ovulation induction via CSF1. *Reprod Biol Endocrinol*. 2020;18(1):26. doi:10.1186/s12958-020-00585-0
50. Li Y, Wu X, Miao S, Cao Q. MiR-383-5p promotes apoptosis of ovarian granulosa cells by targeting CIRP through the PI3K/AKT signaling pathway. *Arch Gynecol Obstet*. 2022;306(2):501-512. doi:10.1007/s00404-022-06461-z
51. Liu Y, Mei Q, Yang J, et al. *hsa-miR-320a-3p* and *hsa-miR-483-5p* levels in human granulosa cells: promising bio-markers of live birth after IVF/ICSI. *Reprod Biol Endocrinol*. 2022;20(1):160. doi:10.1186/s12958-022-01037-7
52. Han S, Zhao X, Zhang Y, et al. MiR-34a-5p promotes autophagy and apoptosis of ovarian granulosa cells via the Hippo-YAP signaling pathway by targeting LEF1 in chicken. *Poult Sci*. 2023;102(2):102374. doi:10.1016/j.psj.2022.102374
53. Fabová Z, Loncová B, Bauer M, Sirotkin AV. Interrelationships between miR-34a and FSH in the control of porcine ovarian cell functions. *Reprod Sci*. 2023;30(6):1789-1807. doi:10.1007/s43032-022-01127-2
54. Cui C, Wang J, Han X, et al. Identification of small extracellular vesicle-linked miRNA specifically derived from intrafollicular cells in women with polycystic ovary syndrome. *Reprod Biomed Online*. 2021;42(5):870-880. doi:10.1016/j.rbmo.2021.02.002
55. Xie S, Batnasan E, Zhang Q, Li Y. MicroRNA expression is altered in granulosa cells of ovarian hyperresponders. *Reprod Sci*. 2016;23(8):1001-1010. doi:10.1177/1933719115625849
56. Ye T, Lin S, Ding S, Cao D, Luo L, Yeung WS. Role of exosomal microRNAs and lncRNAs in the follicular fluid of women with polycystic ovary syndrome. *Res Sq*. 2021:1-15. doi:10.21203/rs.3.rs-720055/v1
57. Nagata S, Inoue Y, Sato T, et al. Age-associated changes in miRNA profile of bovine follicular fluid. *Reproduction*. 2022;164(5):195-206. doi:10.1530/REP-22-0036