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human reproduction update REVIEW

The role of microRNAs in ovarian function and the transition toward novel therapeutic strategies in fertility preservation: from bench to future clinical application

C. Alexandri[1], A. Daniel[1,2], G. Bruylants[3,*], and I. Demeestere[1,4]

[1]Research Laboratory in Human Reproduction, Faculty of Medicine, Université Libre de Bruxelles (ULB), 1070 Brussels, Belgium2Université de Tours, Faculty of Science and Technology, 37200 Tours, France 3Engineering of Molecular NanoSystems, Ecole Polytechnique de Bruxelles, Université Libre de Bruxelles (ULB), 1050 Brussels, Belgium 4Fertility Clinic, CUB-Erasme, 1070 Brussels, Belgium

*Correspondence address. [4]Fertility Clinic, CUB-Erasme, 1070 Brussels, Belgium. E-mail: idemeest@ulb.ac.be orcid.org/0000-0002-3192-6565

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BACKGROUND: New therapeutic approaches in oncology have converted cancer from a certain death sentence to a chronic disease. However, there are still challenges to be overcome regarding the off-target toxicity of many of these treatments. Oncological therapies can lead to future infertility in women. Given this negative impact on long-term quality of life, fertility preservation is highly recommended. While gamete and ovarian tissue cryopreservation are the usual methods offered, new pharmacological-based options aiming to reduce ovarian damage during oncological treatment are very attractive. In this vein, advances in the field of transcriptomics and epigenomics have brought small noncoding RNAs, called microRNAs (miRNAs), into the spotlight in oncology. MicroRNAs also play a key role in follicle development as regulators of follicular growth, atresia and steroidogenesis. They are also involved in DNA damage repair responses and they can themselves be modulated during chemotherapy. For these reasons, miRNAs may be an interesting target to develop new protective therapies during oncological treatment. This review summarizes the physiological role of miRNAs in reproduction. Considering recently developed strategies based on miRNA therapy in oncology, we highlight their potential interest as a target in fertility preservation and propose future strategies to make the transition from bench to clinic.

OBJECTIVE AND RATIONALE: How can miRNA therapeutic approaches be used to develop new adjuvant protective therapies to reduce the ovarian damage caused by cytotoxic oncological treatments?

SEARCH METHODS: A systematic search of English language literature using PubMed and Google Scholar databases was performed through to 2019 describing the role of miRNAs in the ovary and their use for diagnosis and targeted therapy in oncology. Personal data illustrate miRNA therapeutic strategies to target the gonads and reduce chemotherapy-induced follicular damage.

OUTCOMES: This review outlines the importance of miRNAs as gene regulators and emphasizes the fact that insights in oncology can inspire new adjuvant strategies in the field of onco-fertility. Recent improvements in nanotechnology offer the opportunity for drug development using next-generation miRNA-nanocarriers.

WIDER IMPLICATIONS: Although there are still some barriers regarding the immunogenicity and toxicity of these treatments and there is still room for improvement concerning the specific delivery of miRNAs into the ovaries, we believe that, in the future, miRNAs can be developed as powerful and non-invasive tools for fertility preservation.

Key words: oncology / fertility preservation / ovarian protective therapy / microRNAs / nanomedicine

Introduction

The history of the discovery of miRNA dates back to 1993, when lin-4 and, later, let-7 were identified as regulators of developmental timing in *Caenorhabditis elegans* (Lee *et al.*, 1993; Pasquinelli *et al.*, 2000). At that time, miRNAs were thought to be the "dark matter" of the genome; today, almost two decades after their discovery, more than 1000 miRNAs have been detected in all animal species and their importance in gene expression is well recognized (Chen *et al.*, 2018; Li *et al.*, 2010).

MicroRNAs or miRNAs are small non-coding RNA molecules of about 21-22 nucleotides in length and are considered to be major transcriptional and post-transcriptional gene regulators (Wang *et al.*, 2017; Wahid *et al.*, 2010). They are encoded in various intergenic or intragenic genomic regions and are organized either in clusters or encoded individually (de

Rie *et al.*, 2017; Kim and Kim, 2007; Tanzer and Stadler, 2004). The repertoire of miRNA function is rich and well-conserved among animal species (Bartel, 2018).

In this narrative review, we present updated knowledge about ovarian-expressed miRNAs by collecting data via PubMed and Google Scholar databases through to 2019. We highlight the role of miRNAs in ovarian function and disorders and present new perspectives in developing new fertility preservation strategies during cancer treatment based on this breakthrough in oncological therapy.

MicroRNAs synthesis

The location of each miRNA in the genome determines its biogenesis (canonical or non-canonical pathway) (O'Brien *et al.*, 2018a) (Fig 1). According to the canonical pathway, which is the dominant biogenetic process, the miRNA coding region is first transcribed by the RNA Polymerase II enzyme into a long, stem-looped pri-miRNA, and then the microprocessor complex cleaves the stem–loop to release a small hairpin structure called the pre-miRNA (Denli *et al.*, 2004). In the cytoplasm, the pre-miRNA is further cleaved by the ribonuclease type III enzyme, Dicer, to release a small duplex miRNA (Ha and Kim, 2014). Finally, the double stranded miRNA is processed by the RNA-induced silencing complex (RISC). Only one of the two strands, the guide strand, recognizes and binds to the targeted mRNA by perfect or imperfect complementarity leading to mRNA cleavage or translational inhibition (O'Brien *et al.*, 2018a). The other strand, known as the ''passenger'' strand, is often degraded (Rand *et al.*, 2005). It is still unknown whether perfect base-pairing is required for target recognition but it appears that a special interaction between the mRNA binding site architecture and miRNA abundance determine the activity and specificity of the miRNA (Brancati and Großhans, 2018). Consequently, a single miRNA can modulate the expression of several genes with various biological functions and each mRNA can be targeted by several miRNAs with different efficiencies (O'Brien *et al.*, 2018b).

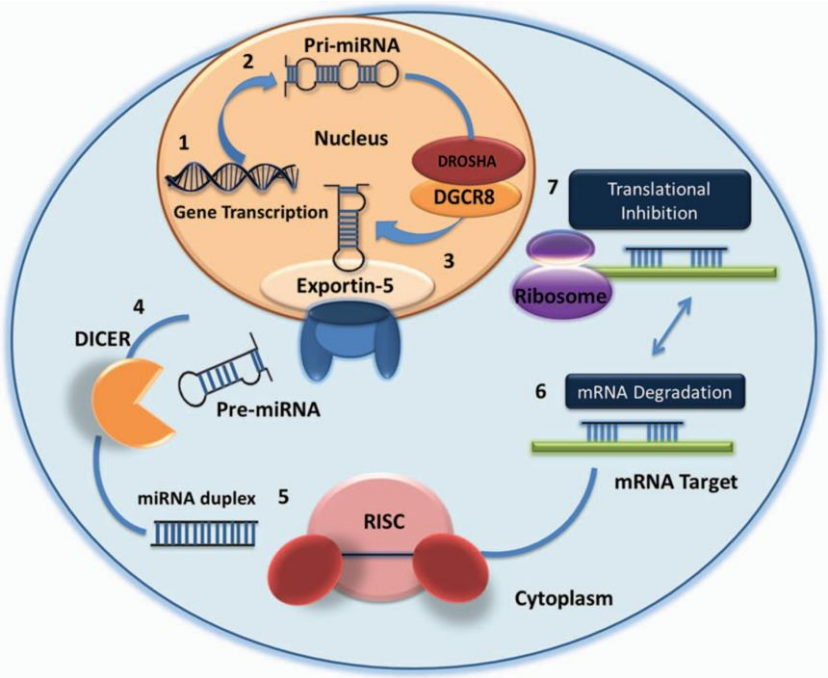

Figure 1 **Canonical pathway of miRNAs biogenesis.** 1: The biogenetic process starts at the nucleus with the transcription of the primary miRNA (pri-miRNA) by RNA polymerase II. 2: The pri-miRNA is a long double stranded molecule with 5-cap, 3-poly-A tail and multiple hairpins,which is processed by DROSHA and DGCR8 to form a precursor-miRNA (pre-miRNA). 3: The pre-miRNA is a stem-looped molecule of ~70 nt, which is exported to cytoplasm by the Ran-GTPase, EXPORTIN 5. 4: DICER removes the stem-loop resulting in the duplex miRNA. 5: Only one of the two strands of the duplex molecule will be incorporated into the RISC complex. This strand is the mature miRNA, named also leading strand, while the other strand (passenger) will be degraded. The functional complex of the mature miRNA and RISC recognizes and binds to the mRNA-target by base pairing. 6: Negative regulation of genes expression by mRNA degradation or 7: Translational inhibition. miRNA: microRNA, DROSHA: class 2 ribonuclease III enzyme, DGCR8: DiGeorge syndrome critical region 8, DICER: double-stranded RNA-specific endoribonuclease, RISC: RNA-induced silencing complex.

MicroRNAs function

MicroRNAs have been characterized as ''molecular rheostats'' and ''fine-tuners'' of gene expression in different tissues and cell types (Bartel, 2009). They can regulate the expression of targeted genes to an extent determined by their abundance and availability (O'Brien *et al.*, 2018b). As miRNA expression is affected by the developmental and health status of the cell, the action of miRNAs highly depends on the type of tissue or organ and its environment. The study of transgenic and miRNA-knockout models has revealed that miRNAs are involved in several biological functions such as development, differentiation, embryogenesis, metabolism, organogenesis, and apoptosis (Hammond, 2015; Ha and Kim, 2014). In addition, miRNAs have an important role in regulating several metabolic pathways and contribute to the maintenance of homeostasis (Vienberg *et al.*, 2017). Moreover, according to recent evidence, it seems that miRNAs participate in cell-to-cell communication (Valadi *et*

al., 2007; Arroyo *et al.*, 2011; Vickers *et al.*, 2011). Hence, extracellular miRNAs may act in a hormone-like manner and play a role as paracrine or endocrine regulators (Iftikhar and Carney, 2016). The plethora of miRNAs and their functions has created the necessity of recording them in online catalogues such as the miRBase database, where more than 38,000 entries of microRNAs from 271 organisms are available (Kozomara *et al.*, 2019). This database is linked to other online platforms such as TargetScan (Agarwal *et al.*, 2015), DIANA-microT (Paraskevopoulou *et al.*, 2013), and miRDB (Wong and Wang, 2015) which use algorithms for miRNA target prediction while TarBase (Karagkouni *et al.*, 2018) and miRTarBase (Chou *et al.*, 2018) include a wide list of experimentally validated miRNA targets. Given the multiple roles of miRNA, these bioinformatics tools are very useful for building miRNA-mRNA interactive networks and for deciphering the signaling pathways that regulate them.

The role of miRNAs in ovarian function

Several studies have illustrated the significant involvement of miRNAs in mammalian ovarian function and reproductive disorders (Santamaria and Taylor, 2014; Sørensen *et al.*, 2014; Bjorkman and Taylor, 2019). The first evidence of the importance of miRNAs in mammalian reproduction was reported after the targeted deletion of enzymes that have a crucial role in miRNA biogenesis like DICER and AGO2 (Nagaraja *et al.*, 2008; Gonzalez and Behringer, 2009; Pastorelli *et al.*, 2009). Transgenic conditional knock-out female mice lacking Dicer1 expression in reproductive tissues presented normal mating behavior but they were infertile due to developmental impairments of the oviduct or uterus (Hong *et al.*, 2008). However, the deficiency of these enzymes had a negative impact on the expression of all miRNAs produced through this biogenetic process. The role of miRNAs in follicle development, oocyte maturation, atresia, steroidogenesis, and luteinization has been extensively reported in previous reviews (Reza *et al.*, 2019; Tesfaye *et al.*, 2018). Therefore, as the list of miRNAs will definitely grow with continual scientific input from research in this field, we present an

overview of these miRNAs as currently described based on the available functionality studies, highlighting their roles in different animal species and in humans (Figure 2).

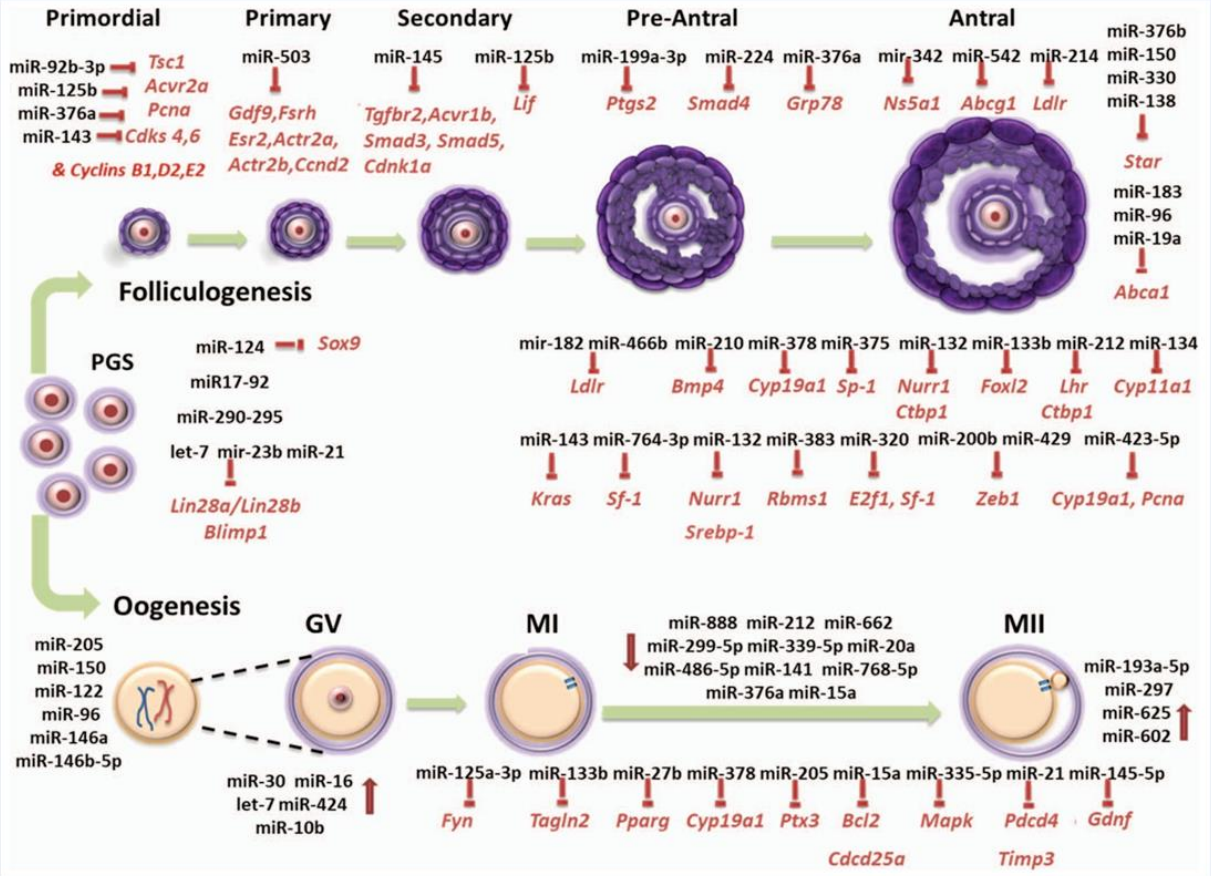

Figure 2 Schematic representation of miRNAs expressed throughout the folliculogenesis and oogenesis. The genes targeted by miRNAs are indicated with red color. PGS: primordial germ cells, GV: germinal vehicle, MI: metaphase I, MII: metaphase II, *Abca1*: ATP-binding cassette transporter ABCA1, *Abcg1*: ATP-binding cassette sub-family g member 1, *Actr2a*: activin receptor type 2a, *Actr2b*: activin receptor type 2b, *Acvr1b*: activin a receptor type 1b, *Acvr2a*: activin receptor type-2a, *Bcl2*: B-cell lymphoma 2, *Blimp1*: b lymphocyte-induced maturation protein-1, *Bmp4*: bone morphogenetic protein 4, *Ccnd2*: cyclin d2, *Cdc25a*: cell division cycle 25 homolog a, *Cdkn1a*: cyclin dependent kinase inhibitor 1a, *Cdks 4,6*: cyclin-dependent kinases 4,6, *Ctbp1*: c-terminal-binding protein 1, *Cyp11a1*: cytochrome P450 family 19 subfamily a member 1, *Cyp19a1*: cytochrome P450 family 19 subfamily a member 1, *E2f1*: steroidogenic factor 1, *Esr2*: estrogen receptor 2, *Foxl2*: forkhead box protein l2, *Fyn*: proto-oncogene tyrosine-protein kinase Fyn, *Gdf9*: growth differentiation factor 9, *Gdnf* : glial cell line-derived neurotrophic factor, *Grp78*: heat shock protein family a (Hsp70) member 5, *Kras*: kirsten rat sarcoma 2 viral oncogene homolog, Ldlr: low-density lipoprotein receptor, *Lhr*: luteinizing hormone receptor, *Lif* : leukemia inhibitory factor, Lin28a: lin-28 homolog a, *Lin28b*: lin-28 homolog b, *Mapk*: mitogen-activated protein kinase, *Nrsa1*: nuclear receptor subfamily 5 group a member 1, *Nurr1:* nuclear receptor related-1 protein, *Pcna:* proliferating cell nuclear antigen, *Pdcd4*: programmed cell death protein 4, *Pparg*: peroxisome proliferator-activated receptor gamma, *Ptgs2*: prostaglandin-endoperoxide synthase 2, *Ptx3*: pentraxin-related protein 3, *Rbms1*: RNA binding motif single stranded interacting protein 1, *Sf-1*: steroidogenic factor 1, *Smad3*: Smad family member 3, *Smad4*: Smad family member 4, *Smad5*: Smad family member 5, *Sox9*: sry-box 9, *Sp-1*: transcription factor 1, *Srebp-1c*: sterol regulatory element-binding transcription factor 1, *Star*: steroidogenic acute regulatory protein, *Tagln2*: transgelin-2, *Tgfbr2*: transforming growth factor-beta receptor type 2, *Timp3*: timp metallopeptidase inhibitor 3, *Tsc1*: tuberous sclerosis 1.

MicroRNAs in follicular development and oocyte maturation

The ovary is a highly dynamic organ characterized by the continual recruitment and development of the follicles which constitute the functional unit. Females are born with a defined number of primordial follicles, also known as the ovarian reserve, which gradually

declines and finally depletes until the onset of menopause (Gleicher *et al.*, 2011). In non-pathological conditions, the balance between follicle dormancy and activation is well-coordinated. MicroRNAs play a special regulatory role in this process by targeting genes involved in folliculogenesis, granulosa cell (GC) proliferation, and oocyte maturation (Tesfaye *et al.*, 2018) (Table 1). In fact, the role of miRNAs in mammalian reproduction starts even earlier at the level of sex determination, where different populations of miRNAs are expressed in primordial germ cells (PGCs). Among them, the overexpression of let-7, miR-125a, and miR-9 PGCs leads to spermatogenesis in mice (Hayashi *et al.*, 2008), while the overexpression of miR-29 induces differentiation in the germ line of female mice (Takada *et al.*, 2009). Others, such as miR-17-92 and the miR-290-295 cluster, act on the migration and colonization of PGCs to the female gonad (Hayashi *et al.*, 2008; Medeiros *et al.*, 2011). After this event, primordial follicle assembly is also modulated by miRNAs. The miR-376a promotes primordial follicle assembly by modulating the expression of the Proliferating cell nuclear antigen (PCNA) which is involved in oocyte apoptosis regulation (Zhang *et al.*, 2014). In mice, miR-143 appears to suppress the proliferation of pregranulosa cells by downregulating the expression of cell cycle-related genes like Cyclin D2, and cyclin-dependent kinases (Cdks) 4 and 6 (Zhang *et al.*, 2013). Other miRNAs that participate in primordial follicle assembly in mouse perinatal ovaries have been described, such as miR-125b which regulates the activin/SMAD2 signaling (Wang *et al.*, 2016). During primordial follicle activation, Yang et al showed that miR-145 regulates the transition of primordial follicles to primary follicles by targeting important genes in the transforming growth factor β (TGF-β) pathway (TGFBR2, Acvr1b, SMAD3 and SMAD5) in mouse ovaries *in vitro* (Yang *et al.*, 2013). According to another study, miR-224 is involved in TGF-β/Smad-mediated granulosa cell proliferation and function by regulating the expression of SMAD4 (Yao *et al.*, 2010). Moreover, miR-92b-3p appears to target Tuberous sclerosis 1 (TSC1) in the mTOR/RPS6 signaling pathway which is essential for primordial follicle recruitment and dormancy as the

upregulation of miR-92b-3p reduced the TSC1 expression leading to mTOR activation and accelerated primordial follicle recruitment (Li *et al.*, 2019). Furthermore, PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a crucial molecule responsible for maintaining the dormancy of the primordial follicles by inhibiting the PI3K/AKT signaling pathway. Its levels require a delicate regulation in order to avoid the massive recruitment of primordial follicles that could lead to premature ovarian reserve depletion. This regulation can be carry out by miRNAs as it has been reported that miR-132, miR-212, and miR-214 and miR-10a target PTEN (Santonocito *et al.*, 2014, Tu *et al.*, 2018). Generally, it is of high interest to decipher the mechanisms of how ovarian follicles remain dormant under such a long period of time before entering into the growing phase. Alongside, we can speculate that miRNAs through the mechanism of gene expression regulation participate in this complex process but their specific role, time and pattern of expression needs to be elucidated. Moreover, it seems that this miRNA and others stimulate prostagladin E2 release in mural GCs, a function that is related to healthy follicular development As GCs proliferate, they create a multi-layer around the oocyte while they start to differentiate into theca cells. Several miRNAs, such as miR-199a-3p, miR-145, miR-31, miR-503, miR-21, and miR-142-3p, were found to be expressed in higher levels in the follicular stage compared to the luteal stage in studies in sheep (McBride *et al.*, 2012). Differences in miRNA expression appear to be related to the selection of the dominant follicle (Tesfaye *et al.*, 2018). Furthermore, studies have demonstrated that miRNAs can coordinate oocyte maturation from prophase I to metaphase II; a multistep and dynamic process characterized by several physiological and morphological transformations (Eppig, 1996; Xu *et al.*, 2011a). Characteristically, miR-27b negatively regulates the peroxisome proliferator activated receptor gamma (PPARγ) which appears to be essential for porcine oocyte maturation (Song *et al.*, 2016), while miR-133b may be implicated in oocyte growth and maturation by targeting Transgelin-2 (TAGLN2) in the Insulin-like growth factor 1 (IGF-1) signaling network (Xiao *et al.*, 2014). Moreover,

several miRNAs present different profiles in the GV (germinal vehicle) and MII (metaphase II) stages, indicating that miRNAs have a specific, rather than global, function in gene expression during oocyte maturation in humans (Xu *et al.*, 2011a; Xu *et al.*, 2011b) and in mice (Cui *et al.*, 2013). Among the identified miRNAs, miR-15a seems to be involved in these processes by targeting B-cell lymphoma 2 (BCL2) and cell division cycle 25A (CDC25A) (Yoon *et al.*, 2009; Solc *et al.*, 2008). It is now clear that a plethora of miRNAs participate throughout the process of folliculogenesis and each developmental stage is characterized by a unique signature of miRNA expression (Reza *et al.*, 2019).

MicroRNAs in steroidogenesis

Follicular steroidogenesis initiates from GCs at the early antral stage with the effects of Follicle Stimulating Hormone (FSH) and, later, Luteinizing Hormone (LH), which are crucial for granulosa cell proliferation, ovulation, and the formation of the corpus luteum. Several studies have demonstrated that miRNAs can regulate the expression of genes involved in steroid hormone production and in turn, the hormonal environment can affect the expression of miRNAs, creating a feedback loop. More specifically, a recent study demonstrated that miR-143 has an important role in granulosa cell proliferation by targeting KRAS (Kirsten rat sarcoma 2 viral oncogene homolog), a key molecule in the MAPK pathway, and also acts as a negative regulator of FSH-induced estradiol production (Zhang *et al.*, 2017). In contrast, FSH decreases miR-143 levels in granulosa cells *in vitro*, leading to decreased aromatase activity and production of estradiol. MicroRNAs such as miR-34a (Sirotkin *et al.*, 2009) and miR-320 (Yin *et al.*, 2014) appear to inhibit the release of estradiol when they are overexpressed in human and mouse granulosa cells. Similarly, miR-764-3p downregulates the expression of steroidogenic factor-1 (SF-1), leading to indirect suppression of CYP19A1 (cytochrome P450 family 19 subfamily A member 1), downstream of Steroidogenic factor 1 (SF-1) (Wang *et al.*, 2016). In addition, other miRNAs positively regulate the release of estradiol. For example, miR-132 (Wu *et al.*, 2015) acts on the cAMP

pathway by targeting Nuclear receptor related-1 protein (NURR1) and induces the expression of CYP19AI, while miR-383 seems to inhibit RBMS1 (RNA-binding motif, single-stranded-interacting protein 1) and repress Cellular myelocytomatosis oncogene (c-MYC) (Yin *et al.*, 2012). Moreover, studies in hypomorphic Dicer1 allele (Dicerd/d) mutant mice revealed that the corpus luteum (CL) requires the proper function of Dicer1 while the lack of miR-17-5p and let-7b led to impaired angiogenesis through the regulation of the antiangiogenic factor tissue inhibitor of metalloproteinase 1 (TIMP-1) (Otsuka *et al.*, 2008). The pleiotropic function of miRNAs is also highlighted by recent study demonstrating a novel regulatory role of miR-210 in CL formation by increasing endothelin-2 (EDN2) expression in hypoxia condition. This study reveals that besides the major role of LH in ovulation and CL formation, hypoxia and hypoxia-modulated miRNAs have also an important role (Shrestha *et al.*, 2018). Last but not least, studies in humans and mice have shown that miRNAs have an endocrine like role in the regulation of the hypothalamic-pituitary axis. Specifically, in double miR-200b and miR-429 knockout mice, the hypothalamic–pituitary–ovarian axis was impaired and the mice failed to ovulate (Hasuwa *et al.*, 2013). Recently, another study in human granulosa cells and serum samples also confirmed that these miRNAs are implicated in the pituitary regulation of human ovulation (Eisenberg *et al.*, 2017) (Figure 2).

MicroRNAs in atresia

In the ovary, most of the primordial follicles remain at the quiescent stage in the stockpile, while a small number are activated and recruited into the growing pool. From these growing follicles, only one will be selected as the dominant follicle in humans. A lot of factors and hormones participate in follicular apoptosis during the atresia process but miRNAs seem to play a crucial regulatory role (Zhang *et al.*, 2019a). Some of the most well-characterized miRNAs include the let-7 family, miR-22, and the miR-23-27-24, miR-183-96-182, and miR-17-92c clusters (Zhang *et al.*, 2019b). In atretic porcine follicles, members of the let-7 family, including let-7a/b/c/I, have been shown to be downregulated, while let-7g was upregulated

(Cao *et al.*, 2015; Zhou *et al.*, 2015a). Moreover, the overexpression of miR-22 has been show to lead to downregulation of the targeted gene NAD-dependent protein deacetylase sirtuin-1 (SIRT1) and to the suppression of apoptosis in mouse granulosa cells (Xiong *et al.*, 2016). In another *in vitro* study, miR-23 and miR-27 have been found to promote FASL-FAS-mediated apoptosis by targeting SMAD5 when they were overexpressed in human granulosa cells (Nie *et al.*, 2015). In contrast, the expression of the miR-183-96-182 cluster has been shown to be increased in dominant follicles (Li *et al.*, 2014; Gebremedhn *et al.*, 2015). The members of this cluster seem to target Forkhead box protein O1 (FOXO1) and hence, control follicular and luteal development by acting on cell survival and steroid production (Mohammed *et al.*, 2017). Several other miRNAs have been shown to participate in follicle atresia regulation by targeting apoptosis effectors like FAS-FASL, Caspases, c-MYC, and TNF, as well as cell survival-promoting factors, such as activin, KIT-KITL (Mast/stem cell growth factor receptor-ligand), IFN (interferon), TP53 (tumour protein P53), and NOBOX (newborn ovary homeobox-encoding gene) (Hussein, 2005) (Table 2).

The role of miRNAs in metabolic and gynecological diseases

MicroRNAs are dysregulated in metabolic-endocrine disorders or gynecological diseases that can affect the ovarian function such as polycystic ovarian syndrome (PCOS), endometriosis or premature ovarian failure (POF) (Supplementary Table 1). Among them, miR-21 has been extensively studied in PCOS and may serve as predictive biomarker as its expression in GCs and follicular fluid was associated with different PCOS patient phenotypes (Naji *et al.*, 2017) and its levels were increased in the serum of PCOS patients (Jiang *et al.*, 2015). Several studies have also implicated miRNAs in the pathogenesis of endometriosis while others have suggested that miRNAs may be useful as diagnostic biomarkers or potential therapeutic targets in this condition (Bjorkman and Taylor, 2019). As an example, Liang et al showed that miR-200c can be used to inhibit the growth of ectopic endometriosis lesions by targeting the RNA N6-adenosine-methyltransferase METTL16 (MALAT1) gene in

cultured human endometrial stromal cells and in mice (Liang *et al.*, 2017). These results suggest that the development of a MALAT1/miR-200c sponge may have therapeutic value in endometriosis.

MicroRNAs have been also involved in premature ovarian failure (POF), as summarized in supplementary table 1. Nevertheless, in most of these cases the specific role of miRNAs has not been elucidated yet.

The aberrant miRNAs expression in several reproductive diseases may be the consequence of the disease or miRNAs dysregulation may be the causative factor. Experimental approaches including *in silico* methodologies or wetlab technics using gain/loss of function miRNA models (animals, cell lines) are needed to elucidate their role or contribution in disease occurrence/progress/monitoring and to identify their targeted genes.

The role of miRNAs in female reproductive cancers

It is well-established that miRNA expression is often dysregulated in several cancer types and this can lead to tumorigenesis and disease progression (Kong *et al.*, 2012). In this setting, miRNAs can either act as tumor suppressors (onco-suppressor-miRs) or as oncogenes (onco-miRs), depending on the nature of the targeted genes and the context of action (Tong and Nemunaitis, 2008; Kasinski and Slack, 2011). Onco-miRs target genes that suppress tumor growth, whereas onco- suppressor-miRs target genes that promote tumorogenesis. Several studies have demonstrated that miRNAs regulate a big network of molecular pathways in carcinogenesis by targeting oncogenes or tumor suppressor genes that are involved in tumor progress and maintenance, angiogenesis, metastasis, drug resistance and cancer-stem-cell biology regulation (Tan *et al.*, 2018).

At present, a big repertoire of onco-miRs and onco-suppressor-miRs have already been established in several cancer types; among them, ovarian, cervical and endometrial cancers. High-throughput techniques such as miRNA microarrays and next generation sequencing

revealed miRNA signatures in ovarian carcinomas like in serous ovarian cancer (Nam *et al.*, 2008) and in stage III/IV epithelial ovarian cancer (Wyman *et al.*, 2009, Iorio *et al.*, 2007). More specifically, in ovarian cancer patients, miR-141, miR-200a, miR-200b, miR-200c have been shown to be upregulated while miR-125b1, miR-140, miR-145, and miR-199a appear to be downregulated compared to healthy tissue (Schwartz *et al.*, 2002). In cervical cancer patients, the pattern of the lowly expressed miR-188 and the highly expressed of miR-223 was correlated with short survival whereas the downregulated profiles of miR-99a and miR-125b were related to the 5-year survival rate (Gao *et al.*, 2018). Characteristically, the downregulation of miR-125b-1 in early stages of cervical cancer seems to inhibit the PI3K/AKT pathway and therefor tumor growth (Granados López and López, 2014; Cui *et al.*, 2012). Moreover, several miRNAs have been reported in endometrial cancer oncogenesis, invasion and metastasis (Yanokura *et al.*, 2015). For example, the miR-30c targets the metastasis-associated gene-1 (MTA1) leading to inhibition of endometrium cell proliferation and hence the downregulation of miR-30c may be involved in endometrial cancer (Hu, 2011). Alongside, let-7a can inhibit the growth of endometrial carcinoma cells by reducing the Aurora-B serine/threonine kinase levels; a protein expressed in normal endometrium cells during the proliferative phase and its expression has been associated with endometrial carcinoma (Liu *et al.*, 2013). In contrast, elucidating the convoluted role of miRNAs in cancer remains a challenge as the reasons for aberrant miRNA expression are still unknown. Recently, emerging data has indicated that the location of miRNAs at fragile chromosomic regions, loss of heterozygosity, and DNA copy number variations may lead to dysregulated miRNA expression profiles (Budhu *et al.*, 2010; Trang *et al.*, 2017; Calin *et al.*, 2004). Moreover, the epigenetic regulation and the localization of miRNAs in the genome determines their regulation at the transcriptional level (Kim and Kim, 2007; Gulyaeva and Kushlinskiy, 2016). It has been shown that in endometrial cancer, the miR-152 is downregulated due to regulation by methylation (Banno *et al.*, 2012). Specifically, miR-152

inhibits the expression of the targeted DNA methyltransferase (DNMT1) which finally affect the methylation process (Tsuruta *et al.*, 2011). In the same way, the hypermethylation of the host gene promoter causes miR-31 silencing in triple-negative breast cancer cell lines (Augoff *et al.*, 2012). Finally, defects in the miRNA biogenesis machinery due to insufficient activity of crucial enzymes, such as Dicer and Drosha, can also affect miRNA expression levels (Gulyaeva and Kushlinskiy, 2016). For example, tumor hypoxia has been associated with reduced expression of Dicer leading to global downregulation of miRNAs in the tumor microenvironment in breast cancer (Rupaimoole *et al.*, 2014) while miRNAs are able to induce tumor hypoxia by regulating Dicer expression. Characteristically, the miR-630 targets Dicer and it is responsible for hypoxia-mediated tumor progression and metastasis as it was detected in invasive epithelial ovarian cancer samples and ovarian and breast cancer cell lines (Rupaimoole *et al.*, 2016). Another paradigm refers to the reciprocal regulation of miRNAs and oncogenes. Members of the miR-34 family are considered to be key regulators of cell cycle, proliferation, and apoptosis, and their function has been found to be downregulated in many malignancies including endometrial cancer (van Rooij and Kauppinen, 2014, Yanokura *et al.*, 2015). It is also important to mention that tumour microenvironment induces further changes in miRNAs expression that further alter the expression of the targeted genes by creating a continual vicious cycle which furher leads to cancer progression.

MicroRNAs modulation by cytotoxic agents

The expression of miRNAs can be also modified by exogenous factors like chemical compounds and cancer treatments (Gulyaeva and Kushlinskiy, 2016). Previous studies have reported that miRNAs are differently expressed in response to cytotoxic agents, such as chemotherapy and radiotherapy, while bioinformatics analyses have revealed that these miRNAs target genes with a key-role in apoptosis and cell proliferation (Phuah *et al.*, 2013; Hummel *et al.*, 2011). Chemotherapeutic drugs like cyclophosphamide, which acts as an

alkylating factor, crosslink DNA, leading to single- or double-strand breaks (Kondo *et al.*, 2010). If the damage is severe, the cell triggers apoptosis. Otherwise, it activates DNA damage response signaling. Ataxia-telangiectasia mutated (ATM) kinase is a key molecule in initiation of the DNA damage response and it is associated with miRNA processing (Tichý *et al.*, 2010). Hence, there are some miRNAs, like miR-16, miR-125b, and miR-199a, whose expression is increased in an ATM-dependent manner (Zhang *et al.*, 2011).

There is emerging interest in the relationship between miRNA expression and chemo- or radio-sensitivity of cancer cells (Yu *et al.*, 2017). For example, miR-214 seems to induce cell survival and cisplatin resistance by acting on PTEN/AKT pathway in human ovarian cancer cell lines (Yang *et al.*, 2008). According to recent studies, various miRNAs are implicated in the development of multiple drug resistance (MDR) in ovarian cancer (Mihanfar *et al.*, 2019). In 2016, Zhao et al. showed that miR-770-5p promoted chemosensitivity by down-regulating ERCC2 (General transcription and DNA repair factor IIH helicase subunit XPD) involved in the DNA damage response (Zhao *et al.*, 2016). Others have reported that overexpression of miR-17-5p induces drug resistance by decreasing the sensitivity of ovarian cancer cells to paclitaxel due to inhibition of caspase 3/7 function and alteration of BAX/BCL-2 expression ratios (Fang *et al.*, 2015).

MicroRNAs stability as potential biomarkers

Except from the endo-cellular miRNAs, a significant number of them has been identified extracellularly, in body fluids like saliva, serum, plasma, follicular fluid and urine (Gilad *et al.*, 2008; Traver *et al.*, 2014a). The extracellularly derived miRNAs, also known as circulating miRNAs may serve as diagnostic and prognostic biomarkers of several diseases, including cancer and gynecological disorders. MicroRNAs are considered ideal candidates for biomarkers as their localization in mini-vehicles like exosomes offers high stability and protection from enzymatic degradation (Traver *et al.*, 2014b). Moreover, miRNAs can be transferred and protected by lipoproteins found in plasma such as HDL (high-density

lipoprotein) and LDL (low-density lipoprotein) (Vickers *et al.*, 2011). Generally, mammalian miRNAs are considered as highly stable molecules but their half-lives are largely unknown (Ji and Chen, 2012). MicroRNAs turnover, is mainly depended on the thermodynamic stability of miRNAs ends while specific motifs at 3' end or in the middle of miRNA's sequence have an important role too (Tomari *et al.*, 2004). According to recent studies, in mammalian cells, there is a miRNA-specific degradation mechanism that controls miRNA activity and viability. There is evidence that a RNA-based mechanism promotes miRNA degradation accompanied by post-transcriptional modifications like tailing, shortening while immunoprecipitation experiments have associated miRNA degradation with Hsp90 co-chaperone Cdc37 (HSP90) or AGO loading (Guo *et al.*, 2015). It seems that the binding of miRNAs to AGO proteins, protects them from degradation but it still remains unclear which mechanism promotes the RISC unloading or degradation. MicroRNAs with half-lives >24 h are characterized as "slow" miRNAs, while "fast" miRNAs present shorter half-lives <12 h (Marzi *et al.*, 2016). Natural modifications to protect miRNAs from rapid degradation include the 2'-O-methylation on the 3' terminal ribose serve as protective mechanisms against 3'-5' degradation and 3' uridylation. Understanding miRNAs turnover is a new focus in the field of miRNA-based approaches and it can help to design more stable molecules.

Despite the fact that each biological fluid (plasma, serum) has a particular miRNA signature, it has been associated with pathological conditions (Gilad *et al.*, 2008). More specifically, in the field of oncology, circulating miRNAs can be useful in cancer early diagnosis, to categorize the different cancer subtypes, to monitor tumor metastasis or to predict the sensitivity of the tumor to oncological treatments (Wang *et al.*, 2018). For example, the miR-186-5p was found to be overexpressed in tumor tissue, blood and urine in bladder cancer patients (He *et al.*, 2017) while breast cancer-related miRNAs (miR-21-5p, miR-125b-5p, miR-155-5p, and miR-451-5p) were differentially expressed in urine samples and they posed a diagnostic potential (Erbes *et al.*, 2015).

Potential therapeutic targets

The identification of miRNA expression levels may have prognostic or predictive value regarding the sensitivity or resistance of cancer cells to the therapeutic regimens (Halimi *et al.*, 2013). Hence, miRNAs can be useful as therapeutic tools to modulate and increase the sensitivity of neoplastic cells to the drugs administered (Hummel *et al.*, 2010). Conversely, miRNAs might be effective for the prediction of off-target toxicity and, hence, to protect healthy cells during oncological treatment. For example, miRNAs can serve as biomarkers of cisplatin-induced cardiotoxicity (Chaudhari *et al.*, 2016) and can be used to protect cardiomyocytes against doxorubicin-induced apoptosis (Tong *et al.*, 2015). Therefore, miRNAs may have a dual role in the management of cancer therapies, acting synergistically with chemotherapy or protectively to reduce off-target toxicity and to minimize chemotherapy side effects. MicroRNA-based therapies can be divided into two main categories: miRNA replacement and miRNA reduction strategies (Chakraborty *et al.*, 2018). A miRNA mimic can be used in order to restore the functionality of the endogenous miRNA which is lost or downregulated. The stability and cell-uptake efficiency of a mimic-miRNA can be increased by using chemically-modified nucleotides or by conjugation with 3'-cholesterol to the passenger strand (van Rooij and Kauppinen, 2014). The first study concerning miRNA replacement strategies was performed in a murine lung cancer model by Esquela-Kerscher and colleagues in 2008 (Esquela-Kerscher *et al.*, 2008). Since then, a lot of studies have moved in this direction and miRNA replacement therapy has gained ground. The first example of a "miRNA-gain-of-function" strategy, which has entered clinical trials (phase I), includes the miR-34 mimic (MRX34). This approach is based on the delivery of MRX34 by a liposome-carrier into the cells. The miRNA-based drug can be injected intravenously into patients with advanced hepatocellular carcinoma but the adverse toxic effects have cast doubt on its safety for clinical applications and this has stopped its entrance to phase II clinical trials (Beg *et al.*, 2017). The toxicity of miRNA-mimic therapeutics is a drawback that

can probably be addressed by optimizing the therapeutic scheme (dosage) and by ameliorating the characteristics of the delivery system (Hosseinahli *et al.*, 2018). Another important miRNA replacement therapy is now in phase I clinical trials and, with promising results, may go through to phase II. This approach is based on the use of the miR-16 mimic in malignant pleural mesothelioma to counterbalance the loss of miR-15/-16, which is responsible for tumor growth progression (van Zandwijk *et al.*, 2017). However, miRNA replacement therapies require optimization in order to avoid supraphysiological levels of the same miRNA and RISC saturation (van Rooij and Kauppinen, 2014).

The inhibition of miRNA function can be facilitated by anti-miRNAs which are small, synthetic molecules with a complementary sequence to the targeted mature miRNA (Rupaimoole and Slack, 2017). There are several anti-miRNA approaches to blocking the function of an over-expressed miRNA and, consequently, to restoring the function of the targeted genes. In order to increase the efficiency of miRNA inhibitors, a series of modifications can be applied such as the sugar modifications or the cholesterol conjugations which have also been applied for miRNA mimic design to improve resistance against to nucleases (van Rooij and Kauppinen, 2014). Moreover, a locked nucleic acid (LNA) structure allows the highest affinity for complementary sequences (Braasch and Corey, 2001, Foss *et al.*, 2017). Moving a step ahead in increasing the stability of miRNA antagonists, miRNA zippers have been developed based on the double miRNA molecule (Meng *et al.*, 2017). Another alternative approach, called miRNA sponges, is under development with the advantage to potentially block an entire miRNA family sharing a similar seed sequence (Ebert *et al.*, 2007). Moving beyond the use of oligonucleotides as modulators of miRNA function, Small Molecular Inhibitors of Specific miRNAs (SMIRs) have also been proposed (Monroig *et al.*, 2015). These molecules can inhibit the generation of miRNAs at different levels (pre-transcriptional, transcriptional, and post-transcriptional) and prevent their maturation (Gumireddy *et al.*, 2008). However, the pathway to SMIR therapy application will be long and complex as specific

characterization of miRNA structure and biogenesis is required (pri-miRNAs, pre-miRNAs, mature miRNAs) (Monroig *et al.*, 2015). Overall, the screening of large chemical libraries to identify lead molecules and drug optimization represent substantial challenging steps towards clinical application.

Female fertility preservation

Over the last few decades, survival from cancer has considerably increased due to significant advances in the field of oncology. As a result, the quality of life in cancer survivors is a primary issue that requires delicate handling. As the classical oncological treatments (radiotherapy, chemotherapy) are not target-specific, they are accompanied by side effects, including the risk of future infertility in young patients (Demeestere *et al.*, 2012). Basically, chemotherapeutic drugs like alkylating agents can cause double-strand DNA breaks in small oocytes and granulosa cells triggering either DNA damage responses or cell death (Soleimani *et al.*, 2011). The damage can occur in both growing and quiescent follicles and leads to the massive recruitment of primordial follicles that is also known as the 'burn out' effect (Meirow *et al.*, 2010). Moreover, cyclophosphamide may directly activate the PI3K pathway, which has been shown to induce follicle activation in synergy with mTORC1 signaling (Roness *et al.*, 2013). Hence, given the negative impact of chemotherapy on ovarian function, fertility preservation is highly recommended.

The remarkable progress in the field of onco-fertility offers young women different fertility preservation options and, therefore, the opportunity to experience pregnancy and motherhood. The fertility preservation approach chosen depends on several parameters, such as patient age, the risk of hormonal stimulation, and delays in starting cancer therapy. The established methods for fertility preservation in adults and post-pubertal girls are oocyte or embryo cryopreservation (Levine, 2014; Coccia *et al.*, 2014). However, this technique is invasive and may interfere with cancer therapeutic schemes (Levine *et al.*, 2010). Another alternative option is ovarian tissue cryopreservation. This is still considered

an experimental technique, but is the only option for fertility preservation in pubertal girls (Demeestere *et al.*, 2010). Besides its invasive aspect, the main concern regarding this method is the risk of cancer relapse after transplantation of the cryopreserved ovarian tissue in cases where there may be remaining malignant cells in the ovarian fragments (Dolmans *et al.*, 2013). Consequently, there are several limitations in the application of these procedures. Therefore, the administration of pharmacological agents concomitantly to chemotherapy that could prevent depletion of the follicle reserve and maintain ovarian function could provide significant advantages over currently existing fertility preservation strategies (Roness *et al.*, 2016).

Current clinical and experimental pharmaco-protective options

The pharmacological protection of the ovaries during oncological treatment is an emerging field in fertility preservation and appears to be very attractive. However, the only pharmacological option clinically tested to reduce treatment-induced gonadotoxicity is the gonadotrophin-releasing hormone analogue (GnRHa). After years of debate, the efficacy of GnRHa remains controversial as the mechanisms of GnRHa ovarian protection have not yet been elucidated (Lambertini *et al.*, 2019). Nevertheless, recent randomized controlled trials have demonstrated the benefit of co-administration of GnRHa during chemotherapy on ovarian function restoration in breast cancer patients but not in lymphoma patients (Rodriguez-Wallberg and Oktay, 2012; Demeestere *et al.*, 2016; Senra *et al.*, 2018).

Several studies assessing new pharmacoprotective strategies have been conducted at the pre-clinical level. The development of agents that can block apoptotic pathways within the ovary, such as sphingosine-1-phosphate (SP1), have been proposed as a promising option (Li *et al.*, 2014a). However, despite the encouraging results of in vivo studies in animals, questions remain regarding the safety of this adjuvant ferto-protective therapy. Likewise, imatinib, which is clinically used for the treatment of chronic myelogenous leukemia, has been proposed as an alternative fertility preservation option. This tyrosine kinase inhibitor

acts on c-Abl, resulting in protection of oocytes during exposure to cisplatin in mice (Morgan *et al.*, 2013; Kim *et al.*, 2013). However, controversial results from other studies have raised questions regarding this protective effect (Kerr *et al.*, 2012). Another anti-cancer drug used in breast cancer, tamoxifen, has been tested in this indication, combined with other anticancer treatments or radiotherapy (Ting and Petroff, 2010; Mahran *et al.*, 2013). A recent in vitro study on rat ovaries suggested that tamoxifen downregulates the expression of several genes involved in chemotherapy-induced inflammation and prevents follicle loss by blocking apoptosis (Piasecka-Srader *et al.*, 2015). However, this effect has yet to be confirmed by further translational studies. In addition, the drug bortezomib (Bort) appears to protect ovaries from doxorubicin in mice by preventing its accumulation in the ovary (Roti Roti *et al.*, 2014). Other approaches aiming to slow down follicular activation have also been tested at the experimental level. Indeed, it has been established that chemotherapeutic drugs like cyclophosphamide and cisplatin can induce primordial follicle activation by triggering the PI3K/PTEN/Akt signaling pathway (Adhikari and Liu, 2009). By acting on this pathway, the immunomodulator AS101 reduces GC apoptosis in growing follicles and the loss of primordial follicles during exposure to cyclophosphamide in mice (Kalechman *et al.*, 1991; Kalich-Philosoph *et al.*, 2013). Moreover, the role of anti-Müllerian hormone (AMH) has been suggested to prevent from follicle activation by inhibiting the recruitment of primordial follicles in treated mice (Sonigo *et al.*, 2019). Recently, Goldman et al showed that the administration of a clinically available mTOR complex 1 inhibitor, everolimus, also maintained the follicular pool and preserved ovarian function by preventing the developmental transition of follicles (primordial to primary) during chemotherapeutic treatment (Goldman *et al.*, 2017). Other approaches that have been proposed for the preservation of fertility during oncological treatment involve drugs acting on blood vessels, such as the Granulocyte Colony-Stimulating Factor (G-CSF) (Skaznik-Wikiel *et al.*, 2013), or on multidrug-resistant transport activity using retrovirus therapy (Brayboy *et al.*, 2013).

However, further studies must be done in order to validate the efficacy and the safety of these options. Last but not least, drug nano-encapsulation is another interesting strategy which has not been investigated yet as far as ovarian protection is concerned. According to this idea, the chemotherapeutic agent would be active only at the site of the tumor, avoiding side effects to other organs. One characteristic example is the encapsulated form of doxorubicin, which reduces the cardiotoxic effects of chemotherapy, and has recently been introduced into clinical use for ovarian cancer and multiple myeloma treatment (Tahover *et al.*, 2015).

Limitations of pharmaco-protective approaches

Despite these impressive breakthroughs in fertility preservation strategies, there are still many limitations regarding the clinical application and safety of these approaches. One of the main conclusions derived from these studies is the difficulty in translating experimental results into clinically available compounds, as there are several barriers regarding the route of administration, specificity, and off-target toxicity. Furthermore, another limitation is that each drug acts on a selected target and, hence, is not effective to prevent all mechanisms involved in ovarian toxicity. Moreover, anti-apoptotic-adjuvant therapies may interfere with cancer therapy efficacy. There is a fragile balance between ‘‘killing’’ cancer cells and ‘‘rescuing’’ ovarian follicles which must lean toward the benefit of the patient.

MicroRNAs therapy in onco-fertility strategies

Considering the limitations of pharmacoprotective drugs, the ideal solution would be to specifically avoid ovarian damage by developing drugs that can target several signaling pathways simultaneously. Given the aforementioned statements, miRNA therapy appears to be a very attractive option in this regard and has already been used to modulate the side effects of anticancer therapies by reducing off-target toxicity. More specifically, miRNAs can be used to orchestrate the expression of genes involved in several pathways which are affected during chemotherapy exposure such as apoptosis, DNA damage response, and cell

proliferation (Figure 3). Moreover, miRNAs have not only tissue-specific but also cell-specific expression profiles which are spatial-temporally dependent. This specificity offers the opportunity for selective targeting (Landgraf *et al.*, 2007). Finally, advances in the field of genomics have revealed that these small non-coding molecules are essential for mammalian ovarian function (Li *et al.*, 2015). The combination of the potential of miRNA therapies and breakthroughs in the field of nanomedicine can lead to the development of new fertility preservation tools with versatile properties.

Preclinical Evidence

Several studies have been conducted in vitro, that have tried to elucidate the role and the wider implications of miRNAs in female reproductive functions and disorders. However, the use of miRNAs as a fertility preservation option has only recently emerged. Evidence regarding the role of miRNAs in chemotherapy-induced ovarian damage was provided by Xiao and colleagues in 2016 when they showed that the delivery of exosome-derived miR-10a and miR-146 in damaged granulosa cells inhibited apoptosis in vitro and prevented follicle atresia in mice in vivo (Xiao *et al.*, 2016). Furthermore, the use of stem cells in the context of fertility preservation seems quite interesting. Fu et al used mesenchymal stem cells (MSCs) as an miR-21 mimic vector to restore ovarian function impaired by chemotherapy exposure in a rat model (Fu *et al.*, 2017). However, it seems that miR-21 is a two-sided sword as far as fertility preservation is concerned. On the one side, the inhibition of PTEN leads to activation of the PI3K pathway and promotes survival by inhibiting apoptosis, but on the other side it can induce the activation of primordial follicles. These experimental findings suggest that miRNAs have an important role in chemotherapy-induced follicle injury which has created new perspectives in fertility preservation strategies. Last but not least, these findings illustrate a different perspective about granulosa cell apoptosis which can be used to favour follicle survival under apoptosis-induced conditions like chemotherapy.

Limitations and Future Perspectives

The field of nanotechnology has demonstrated remarkable growth as a wide range of applications have been reported in medicine, food technology, and chemical production. In nanomedicine, one future perspective relies on the use of gold nanoparticles as an advantageous system for miRNA delivery strategies. The characteristics of gold nanoparticles such as size flexibility, shape plasticity, and the possibility of surface modifications, make them ideal candidates (Dreaden *et al.*, 2012). In addition, repro-toxicological studies are working to elucidate the safety and compatibility of these carriers for use in the female reproductive system (Das *et al.*, 2016). More specifically, the culture of porcine oocytes with various types of gold nanoparticles did not affect their maturation and the microinjection of in vitro cultured murine embryos did not cause toxic effects (Taylor *et al.*, 2015; Taylor *et al.*, 2014). However, the concentration of nanoparticles administered in vivo or used for in vitro studies differs widely from the relevant doses that can be clinically administered (Das *et al.*, 2016). The number of studies that have evaluated the effects of AuNPs on female reproductive organs is very limited, but as soon as the safety of nanoparticles is confirmed, chemically engineered AuNPs can be applied to transfer the selected miRNA molecules directly into the ovary by targeting an ovarian-specific ligand or receptor. Nevertheless, the transition from bench to bedside is complex and requires multiple steps of safety assessment at the preclinical level (Figure 3).

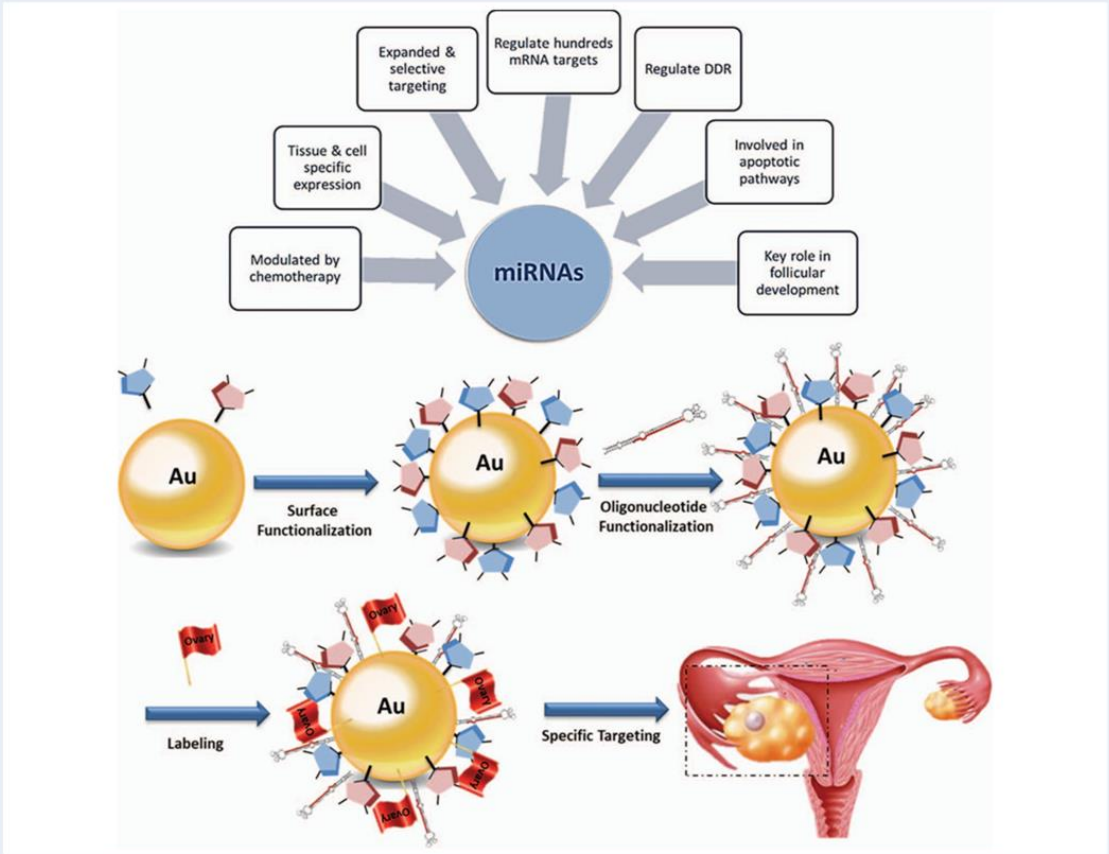

Figure 3 (A) MicroRNAs in fertility preservation concept. (B) Schematic representation of gold nanoparticle (AuNPs) function- alization strategy allowing the further coupling with the miRNA mimics. The manufacturing of surface-engineered gold nanoparticles for targeted miRNA delivery into the ovaries can offer the opportunity for future clinical applications. AuNPs: gold nanoparticles, DDR: DNA damage response.

Limitations of miRNA-based therapeutics

The journey of miRNA therapeutics to reach the targeted organ is long, complex, and unpredictable as they have to face several natural and physiochemical barriers (Chen *et al.*, 2015). They first have to reach the blood stream, avoiding phagocytosis or cleavage by endonucleases. Then, they have to penetrate the negatively charged membrane of the target cell and to exert their function. Finally, the therapeutic applicability of these treatments is determined by their in vivo stability, immunogenicity, and off-target toxicity.

In vivo stability

MicroRNA mimics or antagonists are poly-anionic and water-soluble molecules, with low molecular weights which are ideal for intravenous or subcutaneous injection (Chen *et al.*, 2015). In contrast to endogenous, naturally derived miRNAs (endo-cellular, circulating),

synthetic, unmodified miRNAs are rapidly degraded by RNase A-type nucleases in the systemic circulation (Raemdonck *et al.*, 2008) or subjected to rapid clearance via renal excretion (Yu *et al.*, 2009). The use of a delivery system is required to protect synthetic miRNAs from enzymatic degradation and to facilitate cellular uptake. After cell internalization, chemical modifications on miRNA structures are required to enhance the resistance to endogenous nucleases and increase the miRNA-mRNA hybridization affinity (Seto, 2010).

Immunological response

The systemic delivery of miRNAs can trigger the immune system, resulting in unexpected toxicity and non-desirable side effects (Chen *et al.*, 2015). MicroRNA-mimics or antagonists can trigger different immunogenic responses through the activation of cell surface Toll-like receptors (TLRs) and the release of interferon type I (IFN) or inflammatory cytokines (Singh *et al.*, 2011; Chen *et al.*, 2015). However, the details of the specific immune responses caused by exogenous miRNAs remain unclear. Therefore, an ‘’RNAimmuno database’’ has been created to collect information regarding the nonspecific immunological reactions caused by RNA interference or miRNA modulators (Olejniczak *et al.*, 2010). As the immunogenicity of miRNA therapeutics is a major limitation regarding their clinical application, it has become a research priority to develop more biocompatible and biodegradable miRNA synthetic molecules and delivery systems.

Toxicity

Given that the toxicity of a drug is also based on the dose, the identification of the therapeutic window could limit the occurrence of several side effects. One of these side effects involves the saturation of the RNAi machinery (Chen *et al.*, 2015). MicroRNA therapeutics affect the biogenetic mechanism and action of endogenous miRNAs that can lead to aberrant gene expression and undesirable toxicities. Another important challenge is to reduce off-target effects by enhancing the specificity of organ transfection. Chemical

modifications to the surface of the delivery system can be applied using specific epitopes or receptors in order to reduce the risk of off-target toxicity. Moreover, nanoparticles sized from 15 to 100 nm facilitate systemic delivery and specific miRNA transfer (Brannon-Peppas and Blanchette, 2004). Finally, the method of miRNA-therapeutic administration plays an important role, as the local or topical application of the therapy (skin, eye) can allow for specific targeting to an organ (Chakraborty *et al.*, 2017). Consequently, the chemical properties of the nanoparticle such as size and surface charge, as well as specific modifications relating to the targeted organ or tumor, should be taken into consideration (Pecot *et al.*, 2011).

Nevertheless, the field of miRNA therapeutics has grown substantially over the last five years and the global miRNA market has rapidly gained ground. It is estimated that in 2025, the market's size will be around 626.27 million USD (Ors-Kumoglu *et al.*, 2019). At present, several miRNA-based therapeutics have reached pre-clinical or phase 1 trials.

The challenge of miRNAs delivery systems

Despite the fact that several chemical modifications can be applied in order to increase the stability of synthetic miRNAs, the delivery of ''naked'' oligonucleotides is quite challenging. The natural characteristics of miRNA mimics or antagomirs, their structure and negative charge, make interactions with the negatively-charged cytoplasmic membrane difficult, leading to poor cellular uptake (Bai *et al.*, 2019). Hence, miRNA delivery system should aim to protect the synthetic miRNAs from degradation and facilitate their entrance into host cells. There are several delivery systems available and these can be divided into two large categories: viral and non-viral miRNA carriers (Yang, 2015).

Viral miRNAs delivery

Regarding viral delivery systems, inactivated viral vectors have provided high miRNA-mimic or antagonist transfection efficiencies (Geisler and Fechner, 2016). There are four types of viral vector systems which can be used for miRNA delivery: adenovirus, adeno-associated

virus, retrovirus, and lentivirus. Adenoviruses are internalized by both dividing and non-dividing cells with high efficiency but they can trigger the immune system. Compared to adenoviruses, retrovirus vectors are less immune-reactive but are at high risk of insertional mutagenesis (Herrera-Carrillo *et al.*, 2017). Despite the encouraging results in cell lines and in animal models, the disadvantages of this system have greatly outweighed the advantages as viral transfection can turn into viral infection and genomic mutations leading to toxicity, immunogenicity, and inflammatory response (Yang, 2015).

Non-Viral miRNAs delivery

Non-viral delivery systems have been developed with less negative effects for the host organism but with lower transfection efficiencies compared to viral-delivery systems. These methods use lipid, polymer, and inorganic carriers which are more suitable for clinical applications (Allen and Cullis, 2013; Chan *et al.*, 2010; Lombardo *et al.*, 2019). Lipid-based nanocarriers, also known as liposomes, are mainly cationic and create complexes with negatively-charged nucleic acids like miRNAs. Cell delivery is facilitated by the membrane-like structure of the liposomes and occurs through endocytotic mechanisms (Dominska and Dykxhoorn, 2010). The main disadvantages of this system are the short half-life of the carriers and the non-specific binding to other macromolecules, like serum proteins, in body fluids (Yang, 2015). The polymer-based carriers are mainly composed of polyethyleneimines (PEIs), poly (lactic-co-glycolic) acid (PLGA), and cationic polysaccharides (chitosans, CS). Polyethyleneimines electrostatically interact with the negatively-charged miRNAs and protect the synthetic miRNAs from degradation. They can also escape from the endosomal pathway, which leads to lysosomes (Boussif *et al.*, 1995). However, PEI polymers are non-biodegradable and may interact with other negatively charged molecules leading to toxic effects (Yang, 2015). To overcome this issue, chemical modifications have been applied to increase their biocompatibility, such as PLGAs, which are US FDA-approved (Devulapally and Paulmurugan, 2014). Nevertheless, PLGAs are often targeted by macrophages and they have

low encapsulation efficiency (Bhargava-Shah *et al.*, 2016; Yang, 2015). Another biodegradable polymer, chitosan, is formed by cationic polysaccharides and is produced by chitin but chitosan has low transfection efficiency which limits its application (Mao *et al.*, 2010).

Next-generation miRNA nanocarriers

Recently, a new chapter in the field of nucleic acid delivery has emerged, pushing inorganic carriers to the forefront. Among several inorganic carriers, gold nanoparticles (AuNPs) stand out due to their physicochemical properties. Gold nanoparticles can be modified through surface functionalization for miRNA transfer and specific targeting into cells. Their long half-life and stability enhance miRNA delivery while, at the same time, they do not trigger the immune system and are free of microbial attack (Yang, 2015; Yin, Kanasty *et al.*, 2014). However, the different surface functionalization of AuNPs can change the properties of the particles, creating different toxicity and biocompatible features. Another novel drug-delivery method is based on the use of non-living bacterially-derived minicells (EDVs) (MacDiarmid *et al.*, 2007). This technology has been used to deliver miRNAs to tumors using the ability of EDVs to target cancer cells expressing the EGFR receptor (Reid *et al.*, 2013). Finally, exosomes can also be used as miRNA nanocarriers (Parlea *et al.*, 2016). Exosome-like vesicles can be loaded with the selected miRNA and offer the opportunity for specific cell targeting by surface modifications with selected antibodies against a marker of the target cells (Bryniarski *et al.*, 2013).

Lessons from cancer therapeutics

During the past few years, we have witnessed many milestone discoveries in the field of oncology. The difficulties, the challenges, and the experience obtained from numerous studies has provided valuable background which has elucidated the long path towards innovation in the field of onco-fertility. The common future objective is the development of non-invasive, individualized, and tissue-specific therapy. Today, many miRNA molecules are

being tested in the field of oncology as future therapeutics or adjuvant tools, and second-generation miRNA-nanocarriers provide improved characteristics. Special modifications to the surface of the nanoparticles, like antibody attachment, can offer the opportunity for specific targeting (Brannon-Peppas and Blanchette, 2004). Accordingly, we can speculate that miRNA nanovectors expressing ovarian-derived markers can be efficiently delivered into the organ and protect the ovaries during oncological treatments. One of the most challenging issues in miRNA therapeutic approaches in the field of oncology is to identify the most critical miRNA among the several miRNAs co-expressed in cancerous tumors. Similarly, in the field of onco-fertility, the identification of the miRNA with ovarian protective properties is one of the most laborious tasks to be addressed at the experimental level before proceeding to clinical practice. Although there is still space for research in order to identify the effects that a single miRNA can induce on the cellular level, the list of genes targeted by miRNAs are slowly but surely being decoded. Once these are known, the specific targeting of the ovary and miRNA delivery into ovarian cells poses the same challenge as targeting tumor cells in cancer treatment.

Author's roles

Alexandri C: study design, data collection and analysis, writing and revision of the manuscript. Daniel A: data collection and revision of the manuscript. Demeestere I: study design, data analysis, revision and validation of the final version of the manuscript. All authors approved the final version of the manuscript.

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References

Adhikari D, Liu K. Molecular Mechanisms Underlying the Activation of Mammalian Primordial Follicles. *Endocr Rev* 2009;**30**:438–464.

Agarwal V, Bell GW, Nam J-W, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *Elife* 2015;**4**:e05005.

Allen TM, Cullis PR. Liposomal drug delivery systems: From concept to clinical applications. *Adv Drug Deliv Rev* 2013;**65**:36–48.

Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, *et al.* Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci U S A* 2011;**108**:5003–5008.

Augoff K, McCue B, Plow EF, Sossey-Alaoui K. miR-31 and its host gene lncRNA LOC554202 are regulated by promoter hypermethylation in triple-negative breast cancer. *Mol Cancer* 2012;**11**:5.

Bai Z, Wei J, Yu C, Han X, Qin X, Zhang C, Liao W, Li L, Huang W. Non-viral nanocarriers for intracellular delivery of microRNA therapeutics. *J Mater Chem B* 2019.

Banno K, Kisu I, Yanokura M, Masuda K, Ueki A, Kobayashi Y, Susumu N, Aoki D. Epigenetics and genetics in endometrial cancer: new carcinogenic mechanisms and relationship with clinical practice. *Epigenomics* 2012;**4**:147–162.

Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009;**136**:215–233.

Bartel DP. Metazoan MicroRNAs. *Cell* 2018;**173**:20–51.

Beg MS, Brenner AJ, Sachdev J, Borad M, Kang Y-K, Stoudemire J, Smith S, Bader AG, Kim S, Hong DS. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Invest New Drugs* 2017;**35**:180–188.

Bhargava-Shah A, Foygel K, Devulapally R, Paulmurugan R. Orlistat and antisense-miRNA-

loaded PLGA-PEG nanoparticles for enhanced triple negative breast cancer therapy. *Nanomedicine* 2016;**11**:235–247.

Bjorkman S, Taylor HS. MicroRNAs in endometriosis: biological function and emerging biomarker candidates†. *Biol Reprod* 2019.

Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr JP. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proc Natl Acad Sci U S A* 1995;**92**:7297–7301.

Braasch DA, Corey DR. Locked nucleic acid (LNA): fine-tuning the recognition of DNA and RNA. *Chem Biol* 2001;**8**:1–7.

Brancati G, Großhans H. An interplay of miRNA abundance and target site architecture determines miRNA activity and specificity. *Nucleic Acids Res* 2018;**46**:3259–3269.

Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. *Adv Drug Deliv Rev* 2004;**56**:1649–1659.

Brayboy LM, Oulhen N, Witmyer J, Robins J, Carson S, Wessel GM. Multidrug-resistant transport activity protects oocytes from chemotherapeutic agents and changes during oocyte maturation. *Fertil Steril* 2013;**100**:1428-1435.e7.

Bryniarski K, Ptak W, Jayakumar A, Püllmann K, Caplan MJ, Chairoungdua A, Lu J, Adams BD, Sikora E, Nazimek K, *et al.* Antigen-specific, antibody-coated, exosome-like nanovesicles deliver suppressor T-cell microRNA-150 to effector T cells to inhibit contact sensitivity. *J Allergy Clin Immunol* 2013;**132**:170–181.

Budhu A, Ji J, Wang XW. The clinical potential of microRNAs. *J Hematol Oncol* 2010;**3**:37.

Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, *et al.* Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci* 2004;**101**:2999–3004.

Cao R, Wu WJ, Zhou XL, Xiao P, Wang Y, Liu HL. Expression and Preliminary Functional Profiling of the let-7 Family during Porcine Ovary Follicle Atresia. *Mol Cells* 2015;**38**:304–311.

Chakraborty C, Sharma AR, Sharma G, Doss CGP, Lee S-S. Therapeutic miRNA and siRNA: Moving from Bench to Clinic as Next Generation Medicine. *Mol Ther Nucleic Acids*

2017;**8**:132–143.

Chakraborty C, Sharma AR, Sharma G, Sarkar BK, Lee S-S, Chakraborty C, Sharma AR, Sharma G, Sarkar BK, Lee S-S, *et al.* The novel strategies for next-generation cancer treatment: miRNA combined with chemotherapeutic agents for the treatment of cancer. *Oncotarget* 2018;**9**:10164–10174.

Chan JM, Valencia PM, Zhang L, Langer R, Farokhzad OC. Polymeric Nanoparticles for Drug Delivery. 2010; 163–175.

Chaudhari U, Nemade H, Gaspar JA, Hescheler J, Hengstler JG, Sachinidis A. MicroRNAs as early toxicity signatures of doxorubicin in human-induced pluripotent stem cell-derived cardiomyocytes. *Arch Toxicol* 2016;**90**:3087–3098.

Chen L, Heikkinen L, Wang C, Yang Y, Sun H, Wong G. Trends in the development of miRNA bioinformatics tools. *Brief Bioinform* 2018.

Chen Y, Gao D-Y, Huang L. In vivo delivery of miRNAs for cancer therapy: Challenges and strategies. *Adv Drug Deliv Rev* 2015a;**81**:128–141.

Chen Y, Zhao H, Tan Z, Zhang C, Fu X. Bottleneck limitations for microRNA-based therapeutics from bench to the bedside. *Pharmazie* 2015b;**70**:147–154.

Chou C-H, Shrestha S, Yang C-D, Chang N-W, Lin Y-L, Liao K-W, Huang W-C, Sun T-H, Tu S-J, Lee W-H, *et al.* miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res* 2018;**46**:D296–D302.

Coccia PF, Pappo AS, Altman J, Bhatia S, Borinstein SC, Flynn J, Frazier AL, George S, Goldsby R, Hayashi R, *et al.* Adolescent and Young Adult Oncology, Version 2.2014. *J Natl Compr Cancer Netw* 2014;**12**:21–32.

Cui F, Li X, Zhu X, Huang L, Huang Y, Mao C, Yan Q, Zhu J, Zhao W, Shi H. MiR-125b Inhibits Tumor Growth and Promotes Apoptosis of Cervical Cancer Cells by Targeting Phosphoinositide 3-Kinase Catalytic Subunit Delta. *Cell Physiol Biochem* 2012;**30**:1310–1318.

Cui X-S, Sun S-C, Kang Y-K, Kim N-H. Involvement of microRNA-335-5p in cytoskeleton dynamics in mouse oocytes. *Reprod Fertil Dev* 2013;**25**:691.

Demeestere I, Basso O, Moffa F, Peccatori F, Poirot C, Shalom-Paz E. Fertility preservation in

female cancer patients. *Obstet Gynecol Int* 2012;**2012**:695041.

Demeestere I, Brice P, Peccatori FA, Kentos A, Dupuis J, Zachee P, Casasnovas O, Neste E Van Den, Dechene J, Maertelaer V De, *et al.* No Evidence for the Benefit of Gonadotropin-Releasing Hormone Agonist in Preserving Ovarian Function and Fertility in Lymphoma Survivors Treated With Chemotherapy: Final Long-Term Report of a Prospective Randomized Trial. *J Clin Oncol* 2016;**34**:2568–2574.

Demeestere I, Simon P, Moffa F, Delbaere A, Englert Y. Birth of a second healthy girl more than 3 years after cryopreserved ovarian graft. *Hum Reprod* 2010;**25**:1590–1591.

Denli AM, Tops BBJ, Plasterk RHA, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. *Nature* 2004;**432**:231–235.

Devulapally R, Paulmurugan R. Polymer nanoparticles for drug and small silencing RNA delivery to treat cancers of different phenotypes. *Wiley Interdiscip Rev Nanomedicine Nanobiotechnology* 2014;**6**:40–60.

Dolmans M-M, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. *Fertil Steril* 2013;**99**:1514–1522.

Dominska M, Dykxhoorn DM. Breaking down the barriers: siRNA delivery and endosome escape. *J Cell Sci* 2010;**123**:1183–1189.

Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat Methods* 2007;**4**:721–726.

Eisenberg I, Nahmias N, Novoselsky Persky M, Greenfield C, Goldman-Wohl D, Hurwitz A, Haimov-Kochman R, Yagel S, Imbar T. Elevated circulating micro-ribonucleic acid (miRNA)-200b and miRNA-429 levels in anovulatory women. *Fertil Steril* 2017;**107**:269–275.

Eppig JJ. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. *Reprod Fertil Dev* 1996;**8**:485–489.

Erbes T, Hirschfeld M, Rücker G, Jaeger M, Boas J, Iborra S, Mayer S, Gitsch G, Stickeler E. Feasibility of urinary microRNA detection in breast cancer patients and its potential as an innovative non-invasive biomarker. *BMC Cancer* 2015;**15**:193.

Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L, Weidhaas JB, Brown

D, Bader AG, Slack FJ. The *let-7* microRNA reduces tumor growth in mouse models of lung cancer. *Cell Cycle* 2008;**7**:759–764.

Fang Y, Xu C, Fu Y. MicroRNA-17-5p induces drug resistance and invasion of ovarian carcinoma cells by targeting PTEN signaling. *J Biol Res* 2015;**22**:12.

Foss FM, Querfeld C, Porcu P, Kim YH, Pacheco T, Halwani AS, DeSimone J, William BM, Seto AG, Ruckman J, *et al.* Phase 1 trial evaluating MRG-106, a synthetic inhibitor of microRNA-155, in patients with cutaneous t-cell lymphoma (CTCL). *J Clin Oncol* 2017;**35**:7564–7564.

Gao C, Zhou C, Zhuang J, Liu L, Liu C, Li H, Liu G, Wei J, Sun C. MicroRNA expression in cervical cancer: Novel diagnostic and prognostic biomarkers. *J Cell Biochem* 2018;**119**:7080–7090.

Gebremedhn S, Salilew-Wondim D, Ahmad I, Sahadevan S, Hossain MM, Hoelker M, Rings F, Neuhoff C, Tholen E, Looft C, *et al.* MicroRNA Expression Profile in Bovine Granulosa Cells of Preovulatory Dominant and Subordinate Follicles during the Late Follicular Phase of the Estrous Cycle. In Zhang M, editor. *PLoS One* 2015;**10**:e0125912.

Geisler A, Fechner H. MicroRNA-regulated viral vectors for gene therapy. *World J Exp Med* 2016;**6**:37–54.

Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, *et al.* Serum MicroRNAs Are Promising Novel Biomarkers. In Williams S, editor. *PLoS One* 2008;**3**:e3148.

Gleicher N, Weghofer A, Barad DH. Defining ovarian reserve to better understand ovarian aging. *Reprod Biol Endocrinol* 2011;**9**:23.

Goldman KN, Chenette D, Arju R, Duncan FE, Keefe DL, Grifo JA, Schneider RJ. mTORC1/2 inhibition preserves ovarian function and fertility during genotoxic chemotherapy. *Proc Natl Acad Sci U S A* 2017;**114**:3186–3191.

Gonzalez G, Behringer RR. *Dicer* is required for female reproductive tract development and fertility in the mouse. *Mol Reprod Dev* 2009;**76**:678–688.

Granados López A, López J. Multistep Model of Cervical Cancer: Participation of miRNAs and Coding Genes. *Int J Mol Sci* 2014;**15**:15700–15733.

Gulyaeva LF, Kushlinskiy NE. Regulatory mechanisms of microRNA expression. *J Transl Med* 2016;**14**:143.

Gumireddy K, Young DD, Xiong X, Hogenesch JB, Huang Q, Deiters A. Small-Molecule Inhibitors of MicroRNA miR-21 Function. *Angew Chemie Int Ed* 2008;**47**:7482–7484.

Guo Y, Liu J, Elfenbein SJ, Ma Y, Zhong M, Qiu C, Ding Y, Lu J. Characterization of the mammalian miRNA turnover landscape. *Nucleic Acids Res* 2015;**43**:2326–2341.

Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* 2014;**15**:509–524.

Halimi M, Parsian H, Mohsen Asghari S, Sariri R, Moslemi D, Yeganeh F. MicroRNAs: Are they indicators for prediction of response to radiotherapy in breast cancer? *J Med Hypotheses Ideas* 2013;**7**:59–64.

Hammond SM. An overview of microRNAs. *Adv Drug Deliv Rev* 2015;**87**:3–14.

Hasuwa H, Ueda J, Ikawa M, Okabe M. MiR-200b and miR-429 Function in Mouse Ovulation and Are Essential for Female Fertility. *Science* 2013;**341**:71–73.

Hayashi K, Chuva de Sousa Lopes SM, Kaneda M, Tang F, Hajkova P, Lao K, O'Carroll D, Das PP, Tarakhovsky A, Miska EA, *et al.* MicroRNA Biogenesis Is Required for Mouse Primordial Germ Cell Development and Spermatogenesis. *PLoS One* 2008;**3**:e1738.

He X, Ping J, Wen D. MicroRNA-186 regulates the invasion and metastasis of bladder cancer via vascular endothelial growth factor C. *Exp Ther Med* 2017;**14**:3253–3258.

Herrera-Carrillo E, Liu YP, Berkhout B. Improving miRNA Delivery by Optimizing miRNA Expression Cassettes in Diverse Virus Vectors. *Hum Gene Ther Methods* 2017;**28**:177–190.

Hong X, Luense LJ, McGinnis LK, Nothnick WB, Christenson LK. Dicer1 Is Essential for Female Fertility and Normal Development of the Female Reproductive System. *Endocrinology* 2008;**149**:6207–6212.

Hosseinahli N, Aghapour M, Duijf PHG, Baradaran B. Treating cancer with microRNA replacement therapy: A literature review. *J Cell Physiol* 2018;**233**:5574–5588.

Hu Y. microRNA-30c negatively regulates endometrial cancer cells by targeting metastasis-associated gene-1. *Oncol Rep* 2011.

Hummel R, Hussey DJ, Haier J. MicroRNAs: Predictors and modifiers of chemo- and radiotherapy in different tumour types. *Eur J Cancer* 2010;**46**:298–311.

Hummel R, Wang T, Watson DI, Michael MZ, Hoek M Van der, Haier J, Hussey DJ. Chemotherapy-induced modification of microRNA expression in esophageal cancer. *Oncol Rep* 2011;**26**:1011–1017.

Hussein MR. Apoptosis in the ovary: molecular mechanisms. *Hum Reprod Update* 2005;**11**:162–178.

Iftikhar H, Carney GE. Evidence and potential in vivo functions for biofluid miRNAs: From expression profiling to functional testing. *BioEssays* 2016;**38**:367–378.

Iorio M V., Visone R, Leva G Di, Donati V, Petrocca F, Casalini P, Taccioli C, Volinia S, Liu C-G, Alder H, *et al.* MicroRNA Signatures in Human Ovarian Cancer. *Cancer Res* 2007;**67**:8699–8707.

Ji L, Chen X. Regulation of small RNA stability: methylation and beyond. *Cell Res* 2012;**22**:624–636.

Jiang L, Li W, Wu M, Cao S. Ciculating miRNA-21 as a Biomarker Predicts Polycystic Ovary Syndrome (PCOS) in Patients. *Clin Lab* 2015;**61**:1009–1015.

Kalechman Y, Albeck M, Oron M, Sobelman D, Gurwith M, Horwith G, Kirsch T, Maida B, Sehgal SN, Sredni B. Protective and restorative role of AS101 in combination with chemotherapy. *Cancer Res* 1991;**51**:1499–1503.

Kalich-Philosoph L, Roness H, Carmely A, Fishel-Bartal M, Ligumsky H, Paglin S, Wolf I, Kanety H, Sredni B, Meirow D. Cyclophosphamide Triggers Follicle Activation and Burnout; AS101 Prevents Follicle Loss and Preserves Fertility. *Sci Transl Med* 2013;**5**:185ra62-185ra62.

Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, Vlachos IS, Tastsoglou S, Kanellos I, Papadimitriou D, Kavakiotis I, Maniou S, Skoufos G, *et al.* DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA–gene interactions. *Nucleic Acids Res* 2018;**46**:D239–D245.

Kasinski AL, Slack FJ. Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. *Nat Rev Cancer* 2011;**11**:849–

864.

Kerr JB, Hutt KJ, Cook M, Speed TP, Strasser A, Findlay JK, Scott CL. Cisplatin-induced primordial follicle oocyte killing and loss of fertility are not prevented by imatinib. *Nat Med* 2012;**18**:1170–1172.

Kim S-Y, Cordeiro MH, Serna VA, Ebbert K, Butler LM, Sinha S, Mills AA, Woodruff TK, Kurita T. Rescue of platinum-damaged oocytes from programmed cell death through inactivation of the p53 family signaling network. *Cell Death Differ* 2013;**20**:987–997.

Kim Y-K, Kim VN. Processing of intronic microRNAs. *EMBO J* 2007;**26**:775–783.

Kondo N, Takahashi A, Ono K, Ohnishi T. DNA damage induced by alkylating agents and repair pathways. *J Nucleic Acids* 2010;**2010**:543531.

Kong YW, Ferland-McCollough D, Jackson TJ, Bushell M. microRNAs in cancer management. *Lancet Oncol* 2012;**13**.

Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA sequences to function. *Nucleic Acids Res* 2019;**47**:D155–D162.

Lambertini M, Richard F, Nguyen B, Viglietti G, Villarreal-Garza C. Ovarian Function and Fertility Preservation in Breast Cancer: Should Gonadotropin-Releasing Hormone Agonist be administered to All Premenopausal Patients Receiving Chemotherapy? *Clin Med insights Reprod Heal* 2019;**13**:1179558119828393.

Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993;**75**:843–854.

Levine J, Canada A, Stern CJ. Fertility Preservation in Adolescents and Young Adults With Cancer. *J Clin Oncol* 2010;**28**:4831–4841.

Levine JM. Preserving Fertility in Children and Adolescents with Cancer. *Child* 2014;**1**:166–185.

Li F, Turan V, Lierman S, Cuvelier C, Sutter P De, Oktay K. Sphingosine-1-phosphate prevents chemotherapy-induced human primordial follicle death. *Hum Reprod* 2014a;**29**:107–113.

Li P, Sheng C, Huang L, Zhang H, Huang L, Cheng Z, Zhu Q. MiR-183/-96/-182 cluster is up-

regulated in most breast cancers and increases cell proliferation and migration. *Breast Cancer Res* 2014b;**16**:473.

Li S-C, Chan W-C, Hu L-Y, Lai C-H, Hsu C-N, Lin W. Identification of homologous microRNAs in 56 animal genomes. *Genomics* 2010;**96**:1–9.

Li T, Liu X, Gong X, E Q, Zhang X, Zhang X. microRNA 92b-3p regulates primordial follicle assembly by targeting TSC1 in neonatal mouse ovaries. *Cell Cycle* 2019;15384101.2019.1593648

Liang Z, Chen Y, Zhao Y, Xu C, Zhang A, Zhang Q, Wang D, He J, Hua W, Duan P. miR-200c suppresses endometriosis by targeting MALAT1 in vitro and in vivo. *Stem Cell Res Ther* 2017;**8**:251.

Liu P, Qi M, Ma C, Lao G, Liu Y, Liu Y, Liu Y. Let7a inhibits the growth of endometrial carcinoma cells by targeting *Aurora-B*. *FEBS Lett* 2013;**587**:2523–2529.

Lombardo D, Kiselev MA, Caccamo MT. Smart Nanoparticles for Drug Delivery Application: Development of Versatile Nanocarrier Platforms in Biotechnology and Nanomedicine. *J Nanomater* 2019;**2019**:1–26.

MacDiarmid JA, Mugridge NB, Weiss JC, Phillips L, Burn AL, Paulin RP, Haasdyk JE, Dickson K-A, Brahmbhatt VN, Pattison ST, *et al.* Bacterially Derived 400 nm Particles for Encapsulation and Cancer Cell Targeting of Chemotherapeutics. *Cancer Cell* 2007;**11**:431–445.

Mahran YF, El-Demerdash E, Nada AS, Ali AA, Abdel-Naim AB. Insights into the Protective Mechanisms of Tamoxifen in Radiotherapy-Induced Ovarian Follicular Loss: Impact on Insulin-Like Growth Factor 1. *Endocrinology* 2013;**154**:3888–3899.

Mao S, Sun W, Kissel T. Chitosan-based formulations for delivery of DNA and siRNA. *Adv Drug Deliv Rev* 2010;**62**:12–27.

Marzi MJ, Ghini F, Cerruti B, Pretis S de, Bonetti P, Giacomelli C, Gorski MM, Kress T, Pelizzola M, Muller H, *et al.* Degradation dynamics of microRNAs revealed by a novel pulse-chase approach. *Genome Res* 2016;**26**:554–565.

McBride D, Carré W, Sontakke SD, Hogg CO, Law A, Donadeu FX, Clinton M. Identification of miRNAs associated with the follicular–luteal transition in the ruminant ovary.

REPRODUCTION 2012;**144**:221–233.

Medeiros LA, Dennis LM, Gill ME, Houbaviy H, Markoulaki S, Fu D, White AC, Kirak O, Sharp PA, Page DC, *et al.* Mir-290-295 deficiency in mice results in partially penetrant embryonic lethality and germ cell defects. *Proc Natl Acad Sci* 2011;**108**:14163–14168.

Meirow D, Philosof-Kalich L, Carmely A, Bartal M, Roness H. Follicle "burn out": a novel mechanism of chemotherapy induced ovarian damage. *Fertil Steril* 2010;**94**:S10.

Meng L, Liu C, Lü J, Zhao Q, Deng S, Wang G, Qiao J, Zhang C, Zhen L, Lu Y, *et al.* Small RNA zippers lock miRNA molecules and block miRNA function in mammalian cells. *Nat Commun* 2017;**8**:13964.

Mihanfar A, Fattahi A, Nejabati HR. MicroRNA-mediated drug resistance in ovarian cancer. *J Cell Physiol* 2019;**234**:3180–3191.

Mohammed BT, Sontakke SD, Ioannidis J, Duncan WC, Donadeu FX. The Adequate Corpus Luteum: miR-96 Promotes Luteal Cell Survival and Progesterone Production. *J Clin Endocrinol Metab* 2017;**102**:2188–2198.

Monroig PDC, Chen L, Zhang S, Calin GA. Small molecule compounds targeting miRNAs for cancer therapy. *Adv Drug Deliv Rev* 2015;**81**:104–116.

Morgan S, Lopes F, Gourley C, Anderson RA, Spears N. Cisplatin and Doxorubicin Induce Distinct Mechanisms of Ovarian Follicle Loss; Imatinib Provides Selective Protection Only against Cisplatin. In Franks S, editor. *PLoS One* 2013;**8**:e70117.

Nagaraja AK, Andreu-Vieyra C, Franco HL, Ma L, Chen R, Han DY, Zhu H, Agno JE, Gunaratne PH, DeMayo FJ, *et al.* Deletion of Dicer in somatic cells of the female reproductive tract causes sterility. *Mol Endocrinol* 2008;**22**:2336—2352.

Naji M, Aleyasin A, Nekoonam S, Arefian E, Mahdian R, Amidi F. Differential Expression of miR-93 and miR-21 in Granulosa Cells and Follicular Fluid of Polycystic Ovary Syndrome Associating with Different Phenotypes. *Sci Rep* 2017;**7**:14671.

Nam EJ, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, Kim JW, Kim S. MicroRNA Expression Profiles in Serous Ovarian Carcinoma. *Clin Cancer Res* 2008;**14**:2690–2695.

Nie M, Yu S, Peng S, Fang Y, Wang H, Yang X. miR-23a and miR-27a Promote Human Granulosa Cell Apoptosis by Targeting SMAD51. *Biol Reprod* 2015;**93**:98.

O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front Endocrinol* 2018a;**9**:402.

O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front Endocrinol* 2018b;**9**:402.

Olejniczak M, Galka P, Krzyzosiak WJ. Sequence-non-specific effects of RNA interference triggers and microRNA regulators. *Nucleic Acids Res* 2010;**38**:1–16.

Ors-Kumoglu G, Gulce-Iz S, Biray-Avci C. Therapeutic microRNAs in human cancer. *Cytotechnology* 2019;**71**:411–425.

Otsuka M, Zheng M, Hayashi M, Lee J-D, Yoshino O, Lin S, Han J. Impaired microRNA processing causes corpus luteum insufficiency and infertility in mice. *J Clin Invest* 2008;**118**:1944–1954.

Paraskevopoulou MD, Georgakilas G, Kostoulas N, Vlachos IS, Vergoulis T, Reczko M, Filippidis C, Dalamagas T, Hatzigeorgiou AG. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows. *Nucleic Acids Res* 2013;**41**.

Parlea L, Puri A, Kasprzak W, Bindewald E, Zakrevsky P, Satterwhite E, Joseph K, Afonin KA, Shapiro BA. Cellular Delivery of RNA Nanoparticles. *ACS Comb Sci* 2016;**18**:527–547.

Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Müller P, *et al.* Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* 2000;**408**:86.

Pastorelli LM, Wells S, Fray M, Smith A, Hough T, Harfe BD, McManus MT, Smith L, Woolf AS, Cheeseman M, *et al.* Genetic analyses reveal a requirement for Dicer1 in the mouse urogenital tract. *Mamm Genome* 2009;**20**:140–151.

Pecot C V, Calin GA, Coleman RL, Lopez-Berestein G, Sood AK. RNA interference in the clinic: challenges and future directions. *Nat Rev Cancer* 2011;**11**:59–67.

Phuah NH, In LLA, Azmi MN, Ibrahim H, Awang K, Nagoor NH. Alterations of MicroRNA Expression Patterns in Human Cervical Carcinoma Cells (Ca Ski) toward 1'S-1'-Acetoxychavicol Acetate and Cisplatin. *Reprod Sci* 2013;**20**:567–578.

Piasecka-Srader J, Blanco FF, Delman DH, Dixon DA, Geiser JL, Ciereszko RE, Petroff BK. Tamoxifen Prevents Apoptosis and Follicle Loss from Cyclophosphamide in Cultured Rat

Ovaries1. *Biol Reprod* 2015;**92**

Raemdonck K, Vandenbroucke RE, Demeester J, Sanders NN, Smedt SC De. Maintaining the silence: reflections on long-term RNAi. *Drug Discov Today* 2008;**13**:917–931.

Rand TA, Petersen S, Du F, Wang X. Argonaute2 Cleaves the Anti-Guide Strand of siRNA during RISC Activation. *Cell* 2005;**123**:621–629.

Reid G, Pel ME, Kirschner MB, Cheng YY, Mugridge N, Weiss J, Williams M, Wright C, Edelman JJB, Vallely MP, *et al.* Restoring expression of miR-16: a novel approach to therapy for malignant pleural mesothelioma. *Ann Oncol* 2013;**24**:3128–3135.

Reza AMMT, Choi Y-J, Han SG, Song H, Park C, Hong K, Kim J-H. Roles of microRNAs in mammalian reproduction: from the commitment of germ cells to peri-implantation embryos. *Biol Rev* 2019;**94**:415–438.

Rie D de, Abugessaisa I, Alam T, Arner E, Arner P, Ashoor H, Åström G, Babina M, Bertin N, Burroughs AM, *et al.* An integrated expression atlas of miRNAs and their promoters in human and mouse. *Nat Biotechnol* 2017;**35**:872–878.

Rodriguez-Wallberg KA, Oktay K. Options on fertility preservation in female cancer patients. *Cancer Treat Rev* 2012;**38**:354–361.

Roness H, Gavish Z, Cohen Y, Meirow D. Ovarian follicle burnout: A universal phenomenon? *Cell Cycle* 2013;**12**:3245–3246.

Roness H, Kashi O, Meirow D. Prevention of chemotherapy-induced ovarian damage. *Fertil Steril* 2016;**105**:20–29.

Rooij E van, Kauppinen S. Development of microRNA therapeutics is coming of age. *EMBO Mol Med* 2014;**6**:851–864.

Roti Roti EC, Ringelstetter AK, Kropp J, Abbott DH, Salih SM. Bortezomib prevents acute doxorubicin ovarian insult and follicle demise, improving the fertility window and pup birth weight in mice. *PLoS One* 2014;**9**:e108174.

Rupaimoole R, Ivan C, Yang D, Gharpure KM, Wu SY, Pecot C V, Previs RA, Nagaraja AS, Armaiz-Pena GN, McGuire M, *et al.* Hypoxia-upregulated microRNA-630 targets Dicer, leading to increased tumor progression. *Oncogene* 2016;**35**:4312–4320.

Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov* 2017;**16**:203–222.

Rupaimoole R, Wu SY, Pradeep S, Ivan C, Pecot C V., Gharpure KM, Nagaraja AS, Armaiz-Pena GN, McGuire M, Zand B, *et al.* Hypoxia-mediated downregulation of miRNA biogenesis promotes tumour progression. *Nat Commun* 2014;**5**:5202.

Santamaria X, Taylor H. MicroRNA and gynecological reproductive diseases. *Fertil Steril* 2014;**101**:1545–1551.

Santonocito M, Vento M, Guglielmino MR, Battaglia R, Wahlgren J, Ragusa M, Barbagallo D, Borzì P, Rizzari S, Maugeri M, *et al.* Molecular characterization of exosomes and their microRNA cargo in human follicular fluid: bioinformatic analysis reveals that exosomal microRNAs control pathways involved in follicular maturation. *Fertil Steril* 2014;**102**:1751-1761.

Schwartz DR, Kardia SLR, Shedden KA, Kuick R, Michailidis G, Taylor JMG, Misek DE, Wu R, Zhai Y, Darrah DM, *et al.* Gene expression in ovarian cancer reflects both morphology and biological behavior, distinguishing clear cell from other poor-prognosis ovarian carcinomas. *Cancer Res* 2002;**62**:4722–4729.

Senra JC, Roque M, Talim MCT, Reis FM, Tavares RLC. Gonadotropin-releasing hormone agonists for ovarian protection during cancer chemotherapy: systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2018;**51**:77–86.

Seto AG. The road toward microRNA therapeutics. *Int J Biochem Cell Biol* 2010;**42**:1298–1305.

Shrestha K, Onasanya AE, Eisenberg I, Wigoda N, Yagel S, Yalu R, Meidan R, Imbar T. miR-210 and GPD1L regulate EDN2 in primary and immortalized human granulosa-lutein cells. *Reproduction* 2018;**155**:197–205.

Singh S, Narang AS, Mahato RI. Subcellular Fate and Off-Target Effects of siRNA, shRNA, and miRNA. *Pharm Res* 2011;**28**:2996–3015.

Sirotkin A V., Ovcharenko D, Grossmann R, Lauková M, Mlynček M. Identification of MicroRNAs controlling human ovarian cell steroidogenesis via a genome-scale screen. *J Cell Physiol* 2009;**219**:415–420.

Skaznik-Wikiel ME, McGuire MM, Sukhwani M, Donohue J, Chu T, Krivak TC, Rajkovic A, Orwig KE. Granulocyte colony-stimulating factor with or without stem cell factor extends time to premature ovarian insufficiency in female mice treated with alkylating chemotherapy. *Fertil Steril* 2013;**99**:2045-2054.

Solc P, Saskova A, Baran V, Kubelka M, Schultz RM, Motlik J. CDC25A phosphatase controls meiosis I progression in mouse oocytes. *Dev Biol* 2008;**317**:260–269.

Soleimani R, Heytens E, Darzynkiewicz Z, Oktay K. Mechanisms of chemotherapy-induced human ovarian aging: double strand DNA breaks and microvascular compromise. *Aging* 2011;**3**:782–793.

Song C, Yao J, Cao C, Liang X, Huang J, Han Z, Zhang Y, Qin G, Tao C, Li C, *et al.* PPARγ is regulated by miR-27b-3p negatively and plays an important role in porcine oocyte maturation. *Biochem Biophys Res Commun* 2016;**479**:224–230.

Sonigo C, Beau I, Grynberg M, Binart N. AMH prevents primordial ovarian follicle loss and fertility alteration in cyclophosphamide-treated mice. *FASEB J* 2019;**33**:1278–1287.

Sørensen A, Wissing M, Salö S, Englund A, Dalgaard L, Sørensen AE, Wissing ML, Salö S, Englund ALM, Dalgaard LT. MicroRNAs Related to Polycystic Ovary Syndrome (PCOS). *Genes* 2014;**5**:684–708.

Tahover E, Patil YP, Gabizon AA. Emerging delivery systems to reduce doxorubicin cardiotoxicity and improve therapeutic index. *Anticancer Drugs* 2015;**26**:241–258.

Takada S, Berezikov E, Choi YL, Yamashita Y, Mano H. Potential role of miR-29b in modulation of Dnmt3a and Dnmt3b expression in primordial germ cells of female mouse embryos. *RNA* 2009;**15**:1507–1514.

Tan W, Liu B, Qu S, Liang G, Luo W, Gong C. MicroRNAs and cancer: Key paradigms in molecular therapy. *Oncol Lett* 2018;**15**:2735.

Tanzer A, Stadler PF. Molecular Evolution of a MicroRNA Cluster. *J Mol Biol* 2004;**339**:327–335.

Tesfaye D, Gebremedhn S, Salilew-Wondim D, Hailay T, Hoelker M, Grosse-Brinkhaus C, Schellander K. MicroRNAs: tiny molecules with a significant role in mammalian follicular and oocyte development. *Reproduction* 2018;**155**:R121–R135.

Tichý A, Vávrová J, Pejchal J, Rezácová M. Ataxia-telangiectasia mutated kinase (ATM) as a central regulator of radiation-induced DNA damage response. *Acta medica* 2010;**53**:13–17.

Ting AY, Petroff BK. Tamoxifen decreases ovarian follicular loss from experimental toxicant DMBA and chemotherapy agents cyclophosphamide and doxorubicin in the rat. *J Assist Reprod Genet* 2010;**27**:591–597.

Tomari Y, Matranga C, Haley B, Martinez N, Zamore PD. A Protein Sensor for siRNA Asymmetry. *Science* 2004;**306**:1377–1380.

Tong AW, Nemunaitis J. Modulation of miRNA activity in human cancer: a new paradigm for cancer gene therapy? *Cancer Gene Ther* 2008;**15**:341–355.

Tong Z, Jiang B, Wu Y, Liu Y, Li Y, Gao M, Jiang Y, Lv Q, Xiao X, Tong Z, *et al.* MiR-21 Protected Cardiomyocytes against Doxorubicin-Induced Apoptosis by Targeting BTG2. *Int J Mol Sci* 2015;**16**:14511–14525.

Trang P, Weidhaas JB, Slack FJ. MicroRNAs and Cancer. *Mol Basis Hum Cancer* 2017; 277–286.

Traver S, Assou S, Scalici E, Haouzi D, Al-Edani T, Belloc S, Hamamah S. Cell-free nucleic acids as non-invasive biomarkers of gynecological cancers, ovarian, endometrial and obstetric disorders and fetal aneuploidy. *Hum Reprod Update* 2014a;**20**:905–923.

Traver S, Assou S, Scalici E, Haouzi D, Al-Edani T, Belloc S, Hamamah S. Cell-free nucleic acids as non-invasive biomarkers of gynecological cancers, ovarian, endometrial and obstetric disorders and fetal aneuploidy. *Hum Reprod Update* 2014b;**20**:905–923.

Tsuruta T, Kozaki K -i., Uesugi A, Furuta M, Hirasawa A, Imoto I, Susumu N, Aoki D, Inazawa J. miR-152 Is a Tumor Suppressor microRNA That Is Silenced by DNA Hypermethylation in Endometrial Cancer. *Cancer Res* 2011;**71**:6450–6462.

Tu J, Cheung H-H, Lu G, Chen Z, Chan W-Y. MicroRNA-10a promotes granulosa cells tumor development via PTEN-AKT/Wnt regulatory axis.Cell Death & Disease 2018;**9**:1076.

Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007;**9**:654–659.

Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol* 2011;**13**:423–433.

Vienberg S, Geiger J, Madsen S, Dalgaard LT. MicroRNAs in metabolism. *Acta Physiol (Oxf)* 2017;**219**:346–361.

Wahid F, Shehzad A, Khan T, Kim YY. MicroRNAs: Synthesis, mechanism, function, and recent clinical trials. *Biochim Biophys Acta - Mol Cell Res* 2010;**1803**:1231–1243.

Wang H, Peng R, Wang J, Qin Z, Xue L. Circulating microRNAs as potential cancer biomarkers: the advantage and disadvantage. *Clin Epigenetics* 2018;**10**:59.

Wang L, Li C, Li R, Deng Y, Tan Y, Tong C, Qi H. MicroRNA-764-3p regulates 17β-estradiol synthesis of mouse ovarian granulosa cells by targeting steroidogenic factor-1. *Vitr Cell Dev Biol - Anim* 2016a;**52**:365–373.

Wang S, Liu J, Li X, Ji X, Zhang J, Wang Y, Cui S. MiR-125b Regulates Primordial Follicle Assembly by Targeting Activin Receptor Type 2a in Neonatal Mouse Ovary1. *Biol Reprod* 2016b;**94**.

Wang S, Wu W, Claret FX. Mutual regulation of microRNAs and DNA methylation in human cancers. *Epigenetics* 2017;**12**:187–197.

Wong N, Wang X. miRDB: an online resource for microRNA target prediction and functional annotations. *Nucleic Acids Res* 2015;**43**:D146–D152.

Wu S, Sun H, Zhang Q, Jiang Y, Fang T, Cui I, Yan G, Hu Y. MicroRNA-132 promotes estradiol synthesis in ovarian granulosa cells via translational repression of Nurr1. *Reprod Biol Endocrinol* 2015;**13**:94.

Wyman SK, Parkin RK, Mitchell PS, Fritz BR, O'Briant K, Godwin AK, Urban N, Drescher CW, Knudsen BS, Tewari M. Repertoire of microRNAs in epithelial ovarian cancer as determined by next generation sequencing of small RNA cDNA libraries. *PLoS One* 2009;**4**:e5311.

Xiao G, Xia C, Yang J, Liu J, Du H, Kang X, Lin Y, Guan R, Yan P, Tang S. MiR-133b regulates the expression of the Actin protein TAGLN2 during oocyte growth and maturation: a potential target for infertility therapy. *PLoS One* 2014;**9**:e100751.

Xiong F, Hu L, Zhang Y, Xiao X, Xiao J. miR-22 inhibits mouse ovarian granulosa cell apoptosis by targeting SIRT1. *Biol Open* 2016;**5**:367.

Xu Y-W, Wang B, Ding C-H, Li T, Gu F, Zhou C. Differentially expressed micoRNAs in human oocytes. *J Assist Reprod Genet* 2011a;**28**:559–566.

Xu Y-W, Wang B, Ding C-H, Li T, Gu F, Zhou C. Differentially expressed micoRNAs in human oocytes. *J Assist Reprod Genet* 2011b;**28**:559–566.

Yang H, Kong W, He L, Zhao J-J, O'Donnell JD, Wang J, Wenham RM, Coppola D, Kruk PA, Nicosia S V., *et al.* MicroRNA Expression Profiling in Human Ovarian Cancer: miR-214 Induces Cell Survival and Cisplatin Resistance by Targeting PTEN. *Cancer Res* 2008;**68**:425–433.

Yang N. An overview of viral and nonviral delivery systems for microRNA. *Int J Pharm Investig* 2015;**5**:179–181.

Yang S, Wang S, Luo A, Ding T, Lai Z, Shen W, Ma X, Cao C, Shi L, Jiang J, *et al.* Expression Patterns and Regulatory Functions of MicroRNAs During the Initiation of Primordial Follicle Development in the Neonatal Mouse Ovary. *Biol Reprod* 2013;**89**:1-11,126.

Yanokura M, Banno K, Iida M, Irie H, Umene K, Masuda K, Kobayashi Y, Tominaga E, Aoki D. MicroRNAS in endometrial cancer: recent advances and potential clinical applications. *EXCLI J* 2015;**14**:190–198.

Yao G, Yin M, Lian J, Tian H, Liu L, Li X, Sun F. MicroRNA-224 Is Involved in Transforming Growth Factor-β-Mediated Mouse Granulosa Cell Proliferation and Granulosa Cell Function by Targeting Smad4. *Mol Endocrinol* 2010;**24**:540.

Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. *Nat Rev Genet* 2014a;**15**:541.

Yin M, Lü M, Yao G, Tian H, Lian J, Liu L, Liang M, Wang Y, Sun F. Transactivation of microRNA-383 by steroidogenic factor-1 promotes estradiol release from mouse ovarian granulosa cells by targeting RBMS1. *Mol Endocrinol* 2012;**26**:1129–1143.

Yin M, Wang X, Yao G, Lü M, Liang M, Sun Y, Sun F. Transactivation of MicroRNA-320 by MicroRNA-383 Regulates Granulosa Cell Functions by Targeting E2F1 and SF-1 Proteins. *J Biol Chem* 2014b;**289**:18239–18257.

Yoon S-J, Kim E-Y, Kim YS, Lee H-S, Kim K-H, Bae J, Lee K-A. Role of Bcl2-like 10 (Bcl2l10) in Regulating Mouse Oocyte Maturation1. *Biol Reprod* 2009;**81**:497–506.

Yu B, Zhao X, Lee LJ, Lee RJ. Targeted Delivery Systems for Oligonucleotide Therapeutics. *AAPS J* 2009;**11**:195–203.

Yu X, Chen Y, Tian R, Li J, Li H, Lv T, Yao Q. miRNA-21 enhances chemoresistance to cisplatin in epithelial ovarian cancer by negatively regulating PTEN. *Oncol Lett* 2017;**14**:1807–1810.

Zandwijk N van, Pavlakis N, Kao SC, Linton A, Boyer MJ, Clarke S, Huynh Y, Chrzanowska A, Fulham MJ, Bailey DL, *et al.* Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: a first-in-man, phase 1, open-label, dose-escalation study. *Lancet Oncol* 2017;**18**:1386–1396.

Zhang H, Jiang X, Zhang Y, Xu B, Hua J, Ma T, Zheng W, Sun R, Shen W, Cooke HJ, *et al.* microRNA 376a regulates follicle assembly by targeting Pcna in fetal and neonatal mouse ovaries. *REPRODUCTION* 2014;**148**:43–54.

Zhang J, Ji X, Zhou D, Li Y, Lin J, Liu J, Luo H, Cui S. miR-143 is critical for the formation of primordial follicles in mice. *Front Biosci* 2013;**18**:588–597.

Zhang J, Xu Y, Liu H, Pan Z. MicroRNAs in ovarian follicular atresia and granulosa cell apoptosis. *Reprod Biol Endocrinol* 2019a;**17**:9.

Zhang J, Xu Y, Liu H, Pan Z. MicroRNAs in ovarian follicular atresia and granulosa cell apoptosis. *Reprod Biol Endocrinol* 2019b;**17**:9.

Zhang L, Zhang X, Zhang X, Lu Y, Li L, Cui S. MiRNA-143 mediates the proliferative signaling pathway of FSH and regulates estradiol production. *J Endocrinol* 2017;**234**:1–14.

Zhang X, Wan G, Berger FG, He X, Lu X. The ATM Kinase Induces MicroRNA Biogenesis in the DNA Damage Response. *Mol Cell* 2011;**41**:371–383.

Zhao H, Yu X, Ding Y, Zhao J, Wang G, Wu X, Jiang J, Peng C, Guo GZ, Cui S. MiR-770-5p inhibits cisplatin chemoresistance in human ovarian cancer by targeting ERCC2. *Oncotarget* 2016;**7**:53254–53268.

Zhou J, Liu J, Pan Z, Du X, Li X, Ma B, Yao W, Li Q, Liu H. The let-7g microRNA promotes follicular granulosa cell apoptosis by targeting transforming growth factor-β type 1

receptor. *Mol Cell Endocrinol* 2015;**409**:103–112.

Table I MicroRNAs involved in folliculogenesis, steroidogenesis and oogenesis.

Gene Symbol	Role in Folliculogenesis	Target Genes	Gene Name	Model Species	Reference
miR-124	Sex Determination-Ovarian development	*Sox9*	sry-box 9	mouse	(Real *et al.*, 2013)
let-7 miR-23b miR-21	PGC Specification & Development	*Lin28a/Lin28b & Blimp1*	lin-28 homolog a/b & b lymphocyte-induced maturation protein-1	mouse	(Brieño-Enríquez *et al.*, 2015)
miR-17-92	Essential for Germ Cell Development	unknown		mouse	(Hayashi *et al.*, 2008)
miR-290-295	Essential for Germ Cell Development	unknown		mouse	(Medeiros *et al.*, 2011)
miR-92b-3p	Regulation of Primordial Follicle Assembly	*Tsc1*	tuberous sclerosis 1	mouse	(Li *et al.*, 2019)
miR-125b	Regulation of Primordial Follicle Assembly	*Acvr2a*	activin receptor type-2a	mouse	(Wang *et al.*, 2016)
miR- 376a	Regulation of Primordial Follicle Assembly	*Pcna*	proliferating cell nuclear antigen	mouse	(Zhang *et al.*, 2014)
	Involved in Follicle-Luteal Transition	*Grp78*	heat shock protein family a (Hsp70) member 5	rat	(Iwamune *et al.*, 2014)
miR-503	Expressed in Follicular but not in Luteal Stage	*Gdf9, Fshr, Esr2, Actr2a& Actr2b*	growth differentiation factor 9, follicle stimulating hormone receptor,	mouse	(Lei *et al.*, 2010)

		Ccnd2	estrogen receptor 2, activin receptor type 2a & b, cyclin d2		
miR-143	Expressed in Pre-Granulosa Cells/ Primordial Follicles Formation	*Cdks 4,6 & Cyclins B1, D2, E2* (potential targets)	cyclin-dependent kinases 4,6,	mouse	(Zhang *et al.*, 2013)
miR-145	Primordial Follicles Development & Maintenance	*Tgfbr2, Acvr1b, Smad3, Smad5*	transforming growth factor-beta receptor type 2, activin a receptor type 1b, Smad family member 3,5	mouse	(Yang *et al.*, 2013)
	Involved in Follicle-Luteal Transition	*Cdkn1a*	cyclin dependent kinase inhibitor 1a	cattle	(McBride *et al.*, 2012)
miR-125b	Involved in Follicle-Luteal Transition	*Lif*	leukemia inhibitory factor	cattle	(McBride *et al.*, 2012)
miR-199a-3p	Involved in Follicle-Luteal Transition	*Ptgs2*	prostaglandin-endoperoxide synthase 2	cattle	(McBride *et al.*, 2012)
miR-224	Highly Expressed in Preantral GCs, Promotes Folliculogenesis & GCs Proliferation	*Smad4*	SMAD family member 4	mouse	(Yao *et al.*, 2010)
miR-132 miR-212 miR-214 miR-10a	Primordial Follicle Activation	*PTEN*	phosphatase and tensin homolog	human mouse	(Santonocito *et al.*, 2014, Tu *et al.*, 2018)
Regulation of Steroidogenesis					
Gene Symbol	**Role in Steroidogenesis**	**Target Genes**	**Gene Name**	**Model Species**	**Reference**
miR-143	Mediates the FSH pathway & Regulates Estradiol Production	*Kras*	kirsten rat sarcoma 2 viral oncogene homolog	mouse	(Zhang, Zhang, Zhang, *et al.*, 2017)

miR-764-3p	Regulation of 17β-Estradiol Synthesis	*Sf-1*	steroidogenic factor 1	mouse	(Wang, Li, Li, *et al.*, 2016)
miR-383	Promotes Estradiol Synthesis	*Rbms1*	RNA binding motif single stranded interacting protein 1	mouse	(Yin *et al.*, 2012b)
miR-320	Regulates GCs Proliferation & Steroidogenesis	*E2f1, Sf-1*	transcription factor E2F1, steroidogenic factor 1	mouse	(Yin, Wang, *et al.*, 2014)
miR-200b miR-429	Regulates LH Biosynthesis, Essential for Female Fertility	*Zeb1*	zinc finger E-box-binding homeobox 1	mouse	(Hasuwa *et al.*, 2013)
miR-423-5p	Regulation of Folliculogenic & Steroidogenic Genes Expression	*Cyp19a1, Pcna*	cytochrome P450 family 19 subfamily a member 1, proliferating cell nuclear antigen	pig	(Sui *et al.*, 2014a)
miR-210	Regulation of Folliculogenic & Steroidogenic Genes Expression	*Bmp4*	bone morphogenetic protein 4	pig	(Sui *et al.*, 2014b)
miR-378	Regulation of Folliculogenic & Steroidogenic Genes Expression	*Cyp19a1*	cytochrome P450 family 19 subfamily a member 1	pig	(Sui *et al.*, 2014b)
miR-375	Reduces E2 Synthesis	*Sp-1*	transcription factor 1	pig	(Yu, Li, *et al.*, 2017)
miR-132	Promotes Estradiol Synthesis in GCs	*Nurr1*	nuclear receptor related-1 protein	mouse	(Wu *et al.*, 2015b)
	Hormonal Regulation in Steroid Producing Cells	*Srebp-1c*	sterol regulatory element-binding transcription factor 1	rat	(Hu *et al.*, 2013b)
miR-133b	Stimulates Ovarian Estradiol Synthesis	*FOXL2*	forkhead box protein L2	human, mouse	(Dai *et al.*, 2013)
miR-122	Regulation of LH Receptor	*Lhr*	luteinizing hormone receptor	rat	(Menon *et al.*, 2013)
miR-132 miR-212	Hormonal Regulation at Pre-ovulatory Stage	*Ctbp1* (potential target)	c-terminal-binding protein 1	mouse	(Fiedler *et al.*, 2008)
miR-134	Regulation of Steroidogenesis	*Cyp11a1* (potential	cytochrome P450 family 19 subfamily a member 1	rat	(Hu *et al.*, 2013a)

		target)			
miR-376b miR-150 miR-330 miR-138	Regulation of Steroidogenesis	*Star* *(potential target)*	steroidogenic acute regulatory protein	rat	(Hu *et al.*, 2013b)
miR-342	Regulation of Steroidogenesis	*Nr5a1* (potential target)	nuclear receptor subfamily 5 group a member 1	rat	(Hu *et al.*, 2013b)
miR-182 miR-466b	Regulation of Steroidogenesis	*Ldlr* (potential target)	low-density lipoprotein receptor	rat	(Hu *et al.*, 2013b)
miR-183 miR-96 miR-19a miR-542	Regulation of Steroidogenesis	*Abca1* (potential target)	ATP-binding cassette transporter ABCA1	rat	(Hu *et al.*, 2013b)
miR-542	Regulation of Steroidogenesis	*Abcg1* (potential target)	ATP-binding cassette sub-family g member 1	rat	(Hu *et al.*, 2013b)
miR-214	Hormonal Regulation in Steroid Producing Cells	*Ldlr*	low-density lipoprotein receptor	rat	(Hu *et al.*, 2013b)
miR-17-5p let-7b	Impaired Angiogenesis	*Timp-1*	timp metallopeptidase inhibitor 1	mouse	(Otsuka *et al.*,2008)
miR-210	Hypoxia in Corpus Luteum formation	*EDN2*	endothelin 2	human	(Shrestha *et al.*, 2018)
Regulation of Oogenesis					
Gene Symbol	**Role in Oogenesis**	**Target Genes**	**Gene Name**	**Model Species**	**Reference**
miR-205 miR-150 miR-122, miR-	Highly Expressed in Immature Oocytes	unknown		cattle	(Abd El Naby *et al.*, 2013)

96 miR-146a miR-146b-5p					
miR-30 miR-16 let-7	Highly Expressed in GV Oocytes	unknown		mouse	(Murchison *et al.*, 2007)
miR-424 miR-10b	Abundant in GV Oocytes Role in Zygotic Genome Activation	unknown		cattle	(Tripurani *et al.*, 2010)
miR-125a-3p	Regulation of GVBD	*Fyn*	proto-oncogene tyrosine-protein kinase Fyn	mouse	(Grossman *et al.*, 2017)
miR-133b	Oocyte Growth & Maturation	*TAGLN2*	transgelin-2	human/ mouse	(Xiao *et al.*, 2014)
miR-27b	Role in Oocyte Maturation	*Pparg*	peroxisome proliferator-activated receptor gamma	pig	(Song *et al.*, 2016)
miR-378	Regulation of Oocyte Maturation	*Cyp19a1*	cytochrome P450 family 19 subfamily a member 1	pig	(Pan *et al.*, 2015)
	Regulation of Oocyte Maturation	*unknown*		mouse	(Sun *et al.*, 2018)
miR-205	BDNF-Induced Oocyte Maturation	*Ptx3*	pentraxin-related protein 3	pig	(Li *et al.*, 2016)
miR-15a	Oocyte Growth & Maturation	*BCL2, CDC25A*	b-cell lymphoma 2, cell division cycle 25 homolog a	human	(Xu *et al.*, 2011b)
miR-335-5p	Involvement in Cytoskeleton Dynamics	*Mapk*	mitogen-activated protein kinase	mouse	(Cui *et al.*, 2013)
miR-21	Differentially Expressed During Meiotic Maturation	*Pdcd4*	programmed cell death protein 4	pig	(Wright *et al.*, 2016)
	Upregulation of Metalloprotease during COC in vitro maturation	*Timp3*	metalloproteinase inhibitor 3	pig	(Bo Pan and Julang Li, 2018)
miR-145-5p	Downregulation Contributes to Oocyte Maturation & Cell Viability	*Gdnf*	glial cell line-derived neurotrophic factor	human	(Cui *et al.*, 2018)

miR-193a-5p miR-297 miR-625 miR-602	Upregulated in MII Oocyte Compared to GV Oocytes	unknown		human	(Xu *et al.*, 2011b)
miR-888 miR-212 miR-662 miR-299-5p miR-339-5p miR-20, miR-486-5p miR-141 miR-768-5p miR-376a miR-15a	Downregulated in MII-Stage Compared to GV Oocytes	unknown		human	(Xu *et al.*, 2011b)

miRNA: PGC: primordial germ cells, LH: luteinizing hormone, E2: prostaglandin, GCs: granulosa cells GV: germinal vesicle, GVBD: germinal vesicle breakdown, BDNF: brain-derived neurotrophic factor, COC: cumulus oocyte complex, MII: metaphase II

Table II miRNAs involved in follicle apoptosis and atresia in GCs.

Gene Symbol	Function: GCs Apoptosis/ Atresia	Target Genes	Gene Name	Model Species	Reference
miR-141-3p	Apoptosis Inhibition	*Dapk1*	death associated protein kinase 1	rat	(Li *et al.*, 2017)
miR-26b	Pro-apoptotic Factor	*Smad4*	Smad family member 4	pig	(Liu, Du, *et al.*, 2014)
	Apoptosis Induction	*Atm*	ataxia telangiectasia mutated	pig	(Lin *et al.*, 2012)
	Apoptosis Induction	*Has2*	hyaluronan synthase 2	pig	(Liu, Tu, *et al.*, 2016)
miR-126-3p	Promotes Proliferation & Inhibits Apoptosis	*Tsc1*	tuberous sclerosis 1	pig	(Yuan *et al.*, 2018)
miR-125a-5p	Apoptosis Induction	*Stat3*	signal transducer and activator of transcription 3	mouse	(Wang, Li, Zhang, *et al.*, 2016)
miR-125b	Atresia	*unknown*		mouse	(Sen *et al.*, 2014)
	Apoptosis Induction	*Bmpr1b*	bone morphogenetic protein receptor type 1b	yak	(Yao, Niu, *et al.*, 2018)
miR-145	Protects from Oxidative Induced Apoptosis	*Klf4*	kruppel-like factor 4	mouse	(Xu *et al.*, 2017)
let-7g	Apoptosis Induction	*Map3k1*	mitogen-activated protein kinase kinase kinase 1	pig	(Cao, Wu, Zhou, Liu, *et al.*, 2015)

	Apoptosis Induction	*Tgbr1*	transforming growth factor beta receptor I	pig	(Zhou *et al.*, 2015b)
	Regulates Autophagy	*Igfr1*	insulin-like growth factor 1	mouse	(Zhou, Yao, *et al.*, 2016)
	Increased in Atresia	unknown		pig	(Cao, Wu, Zhou, Xiao, *et al.*, 2015)
let-7a/b/c/i	Decreased in Atresia	*unknown*		pig	(Cao, Wu, Zhou, Xiao, *et al.*, 2015)
miR-34a	Apoptosis Induction	*Inhbb*	inhibin, beta b	pig	(Tu *et al.*, 2014)
miR-34c	Pro-apoptotic Factor	unknown		pig	(XU *et al.*, 2016)
miR-92a	Apoptosis Inhibition	*Smad7*	Smad family member 7	pig	(Liu, Yao, *et al.*, 2014)
miR-27a	Apoptosis Induction	*SMAD5*	Smad family member 5	human	(Nie *et al.*, 2015)
miR-23a	Apoptosis Induction	*SMAD5*	Smad family member 5	human	(Nie *et al.*, 2015)
	Apoptosis Induction	*BCL-2*	b-cell lymphoma 2	human	(Luo *et al.*, 2019)
miR-22	Apoptosis Inhibition	*Sirt1*	NAD-dependent deacetylase sirtuin-1	mouse	(Xiong *et al.*, 2016)
miR- 21	Apoptosis Inhibition	*unknown*		mouse	(Carletti *et al.*, 2010)
	Apoptosis Inhibition	*Smad7*	Smad family member 7	rat	(Zhang, Gao, and Cui, 2017)
miR-10 family	Proliferation Suppression & Apoptosis	*BDNF*	brain-derived neurotrophic factor	human/rat/mouse	(Jiajie *et al.*, 2017)

	induction				
miR-10b	Proliferation Suppression	*Bdnf*	brain-derived neurotrophic factor	goat	(Peng *et al.*, 2016)
miR-146a	Apoptosis Induction	*IRAK1/TRAF6*	Interleukin-1 receptor-associated kinase 1/ tumor necrosis factor receptor associated factor 6	human	(CHEN *et al.*, 2015)
miR-181a	Apoptosis Induction	*Sirt1*	NAD-dependent deacetylase sirtuin-1	mouse	(Zhang, Zhang, Hu, *et al.*, 2017)
miR-181b	Apoptosis Induction	*Smad7*	Smad family member 7	pig	(Yao, Pan, *et al.*, 2018)
miR-224	Apoptosis Induction	*Smad4*	Smad family member 5	mouse	(Yao *et al.*, 2010)
miR-150	Apoptosis Induction	*Star*	steroidogenic acute regulatory protein	sheep	(Zhou *et al.*, 2019)
miR-1275	Promotes Early Apoptosis & Atresia Initiation	*Lrh-1*	luteinizing hormone receptor	pig	(Liu, Li, *et al.*, 2018)
miR-15a miR-96 miR-92 miR-124 miR-18 miR-29a miR-125a miR-136 miR-147 miR-183 miR-32	Apoptosis Induction	*BAX*	bcl-2-associated X protein	human	(Sirotkin *et al.*, 2010)
miR-21-5p miR-21-3p miR-150	Higher Levels in Atretic than Healthy	*Hif1a*, *Vegfa*, *Ets1*, *Msh2*	hypoxia-inducible factor 1-alpha, vascular endothelial	cattle	(Donadeu *et al.*, 2017)

miR-409a, miR-142-5p miR-378 miR-222 miR-155 miR-199a-5p	Follicles		growth factor a, ets proto-oncogene 1, muts homolog 2		

GCs: granulosa cells

Supplementary Table SI Potential role of the main miRNAs reported in metabolic and gynaecological diseases.

miRNAs Involved in PCOS					
Gene Symbol	**Function in PCOS**	**Target Genes**	**Gene Name**	**Model Species**	**Reference**
miR-323-3p	Regulation of Steroidogenesis & Apoptosis	*IGF-1*	insulin growth factor 1	human	(Wang *et al.*, 2019)
miR-142 miR-33b miR-423	Dysregulated in GCs	*TGFBR1 & SMAD7*	transforming growth factor-beta 1 & SMAD family member 7	human	(Li *et al.*, 2019)
miR-222	Promotes PCOS Progression	*p27*	cyclin-dependent kinase inhibitor 1b	human	(Huang *et al.*, 2019)
miR-324-3p	Overexpression Decreases Proliferation & Induces GCs Apoptosis	*Wnt2b*	wingless-type MMTV integration site family member 2	rat	(Jiang and Ma, 2018)
miR-99a	Regulates Proliferation & Apoptosis of GCs in PCOS	*IGF-1R*	insulin growth factor 1 receptor	human	(Geng *et al.*, 2019)
miR-16	Promotes GCs Proliferation & Suppresses Apoptosis	*PDCD4*	programmed cell death 4	human	(Fu *et al.*, 2018)
miR-483-5p miR-486-5p	Downregulated in Cumulus cells of Metaphase II Oocytes	unknown		human	(Shi *et al.*, 2015)
miR-141 miR-200c	Overexpressed in GCs	unknown		human	(He *et al.*, 2018)
miR-19b	Promotes GCs proliferation	*IGF-1*	insulin growth factor 1	human	(Zhong *et al.*, 2018)
miR-33b-5p	Overexpressed in PCOS & Inhibits Glut4	*Hmga2*	high-mobility group AT-hook 2	rat	(Yang *et al.*, 2018)

miR-15a	Dysregulated in PCOS & Involvement in pathogenesis	*unknown*		human	(Naji *et al.*, 2018)
miR-182	Dysregulated in PCOS & Involvement in pathogenesis	*unknown*		human	(Naji *et al.*, 2018)
miR-320a	Impaired Steroidogenesis in cumulus GCs	*RUNX2*	runt-related transcription factor 2	human	(Zhang *et al.*, 2017)
miR-146a	Inflammation & Oxidative stress in PCOS	unknown		mouse	(Salimi-Asl *et al.*, 2016)
miR-509-3p	Promotes Oestradiol Secretion	*MAP3K8*	mitogen-activated protein kinase kinase kinase 8	human	(Huang *et al.*, 2016)
miR-592	Downregulated in PCOS	*LHCGR*	luteinizing hormone/choriogonadotropin receptor	human	(Song *et al.*, 2015)
miR-27a-3p	Affects Estradiol & Androgen Imbalance in GCs	*Creb1*	cAMP responsive element binding protein 1	mouse	(Wang *et al.*, 2018)
miR-93	Increased Expression in PCOS- Diagnostic Biomarker	unknown		human	(Sathyapalan *et al.*, 2015)
	Differential Expression in GCs & Follicular Fluid- Associating with Different PCOS Phenotypes	Genes in TGFB signaling pathway (potential targets)	transforming growth factor beta pathway	human	(Naji *et al.*, 2017)
	Promotes GCs Proliferation	*CDKN1A*	cyclin dependent kinase inhibitor 1a	human	(Jiang *et al.*, 2015)
miR-21	Predictive Biomarker in PCOS	*LATS1*	large tumor suppressor kinase 1	human	(Jiang *et al.*, 2015)
	Inflammation & Oxidative stress in PCOS	unknown		mouse	(Salimi-Asl *et al.*, 2016)

	Differential Expression in GCs & Follicular Fluid- Associating with Different PCOS Phenotypes	Genes in TGFB signaling pathway (potential targets)	transforming growth factor beta	human	(Naji *et al.*, 2017)
miR-145	Negative Regulation of GC Proliferation	*IRS1*	insulin receptor substrate 1	human	(Cai *et al.*, 2017)
	Dysregulated in PCOS & Involvement in pathogenesis	*unknown*		human	(Naji *et al.*, 2018)
miRNAs Involved in Endometriosis					
Gene Symbol	**Function in Endometriosis**	**Target Genes**	**Gene Name**	**Model Species**	**Reference**
miR-2861	Proliferation & Apoptosis Regulation in ectopic ESCs	*STAT3 & MMP2*	signal transducer and activator of transcription 3 & matrix metallopeptidase 2	human	(Yu *et al.*, 2019)
miR-488	Inhibition of endometrial glandular epithelial cell proliferation, migration & invasion	*Wnt* Signaling/ *Fzd7ZD7*	wingless-INT signaling/ frizzled class receptor 7	mouse	(Zhu *et al.*, 2019)
miR-370-3p	Regulates cell proliferation in ESCs	*SF-1*	steroidogenic factor 1	human	(Hu *et al.*, 2019)
miR-138	Induces exosome-mediated inflammation & apoptosis in ESCs	*Vegf/Nfκb* signaling pathway	Vascular endothelial growth factor/nuclear factor kappa-light-chain-enhancer of activated B cells	rat	(Zhang *et al.*, 2018)
miR-221	Upregulated in ectopic endometrial tissues & ectopic endometrial stroma cells	*PTEN* (potential target)	phosphatase and tensin homolog	human	(Du *et al.*, 2018)
miR-363	Overexpression inhibited the invasion ability of ESCs	unknown		human	(Li *et al.*, 2018)
miR-449b-3p	Overexpression inhibited the proliferation of ESCs	unknown		human	(Liu, Chen, *et al.*, 2018)

miR-135a/b	Increased Expression in endometriosis	*HOXA10*	homeobox protein Hox-A10	human	(Petracco *et al.*, 2011)
miR-199a	Downregulation, Attenuates ESCs invasiveness	*IKKB/NF-kB pathway, IL-8*	Inhibitor of nuclear factor kappa-B kinase subunit beta/ nuclear factor kappa-light-chain-enhancer of activated B cells, interleukin-8	human	(Dai *et al.*, 2012)
miR-126	Decreased expression may play an initial role in endometriosis	*CRK*	proto-oncogene c-Crk	human	(Liu *et al.*, 2012)
miR-17-5p miR-20a miR-22	Downregulation in the plasma in women with endometriosis	unknown		human	(Jia *et al.*, 2013)
miR-194-3p	Overexpressed in the eutopic endometrium, decreased progesterone signaling, decidualization defects	*PR*	progesterone receptor	human	(Pei *et al.*, 2018)
miR-214	Upregulation may inhibit fibrogenesis	unknown		human /mouse	(Wu *et al.*, 2018)
miR-200c	Endometriosis Suppression	*MALAT1*	metastasis associated lung adenocarcinoma transcript 1	human /rat	(Liang *et al.*, 2017)
miR-34a-5p	Contribution in Endometriosis Pathogenesis	*VEGFA*	vascular endothelial growth factor a	human	(Ma *et al.*, 2017)
miR-503	Epigenetically Repressed in Endometriosis, Induces Apoptosis, Cell-cycle Arrest, Inhibits Cell Proliferation, Angiogenesis & Contractility of ESCs	*CDK1, VEGFA*	cyclin dependent kinase 1, vascular endothelial growth factor a	human	(Hirakawa *et al.*, 2016)
miR-29c	Progesterone Resistance	*FKBP4*	FK506-binding protein 4	human	(Long *et al.*, 2015)
	Downregulated in the Ectopic Endometrium Affects ECs Proliferation, Apoptosis & Invasion	*C-JUN*	jun proto-oncogene	human	(Long *et al.*, 2015)

miR-196a	Potential Biomarker of Endometriosis	*MEK/ERK* signaling	mitogen-activated protein kinase / extracellular signal-regulated kinase	human	(Zhou *et al.*, 2016)
miRNA-15a-5p	Contribution in Endometriosis pathogenesis	*VEGFA*	vascular endothelial growth factor a	human	(Liu *et al.*, 2016)
let-7f	Induced Migration of ECs	*CYP19A1*	cytochrome P450 family 19 subfamily a member 1	human	(Cho *et al.*, 2016)
miR-200b	Affects ECs Proliferation, Invasiveness & Stemness	*ZEB1, ZEB2, KLF4*	zinc finger E-box binding homeobox 1/2, kruppel like factor 4	human	(Eggers *et al.*, 2016)
miR-183	Promotes Invasion of ECs	*ITGB1P*	integrin b1	human	(Shi *et al.*, 2014)
miR-93	Downregulation Contributes to Endometriosis	*MMP3, VEGFA*	matrix metallopeptidase 3/ vascular endothelial growth factor a	human	(Lv *et al.*, 2015)
miR-142-3p	Regulation of Cell Viability & Proinflammatory Signalling in ECs	*IL-6*	Interleukin 6	human	(Kästingschäfer *et al.*, 2015)
miR-210	Enhanced Expression Promotes Endometriosis Pathogenesis	*STAT3*	signal transducer and activator of transcription 3	human	(Okamoto *et al.*, 2015)
miR-20a	Contributes to Endometriosis	*NTN4*	netrin 4	human	(Zhao *et al.*, 2014)
miR-451	Impairs Endometrial Tissue Ability to Establish Ectopically	*Fga*	fibrinogen alpha chain	mouse	(Nothnick *et al.*, 2014)
miRNAs Involved in POF					
Gene Symbol	**Function in (POF)**	**Target Genes**	**Gene Name**	**Model Species**	**Reference**

miR-146a	Downregulation Inhibits GCs Apoptosis	*IRAK1, TRAF6*	interleukin 1 receptor associated kinase 1, TNF receptor associated factor 6	human	(CHEN *et al.*, 2015)
miR-379-5p	Overexpression Inhibited GC Proliferation & Attenuated DNA Repair Efficiency	*PARP1, XRCC6*	poly(ADP-ribose) polymerase 1, X-ray repair cross complementing 6	human	(Dang *et al.*, 2018)
miR-15a	Involved in POF Progression via Hippo-YAP/TAZ signaling	*Lats1*	large tumor suppressor kinase 1	mouse	(Ai *et al.*, 2018)
miR-22-3p	Downregulated in the Plasma of POF patients	unknown		human	(Guo *et al.*, 2017)
mir-23a	Potential Regulation GCs Apoptosis	*XIAP*	X-linked inhibitor of apoptosis	human	(Yang *et al.*, 2012)
miR-29a miR-144	Downregulated in POF - Regulate Prostaglandin Biosynthesis	*Pla2g4a*	phospholipase a2 group IVA	rat	(Kuang *et al.*, 2014)

PCOS: polycystic ovary syndrome, POF: premature ovarian failure, ECs: endometrial cells ESC: endometriotic stromal cells GC: granulosa cell, Glut4: glucose transporter type 4, YAP/TAZ: Yes-associated protein/transcriptional co-activator with PDZ-binding motif (TAZ)