The role of micro-RNAs in the female reproductive tract

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Abstract

Proper development and function of the female reproductive tract are essential for successful reproduction. Regulation of the differentiated functions of the organs that make up the female reproductive tract is well established to occur at multiple levels including transcription, translation, and posttranslational modifications. Micro-RNA (miRNA)-mediated posttranscriptional gene regulation has emerged as a fundamental mechanism controlling normal tissue development and function. Emerging evidence indicates that miRNAs are expressed within the organs of the female reproductive tract where they function to regulate cellular pathways necessary for proper function of these organs. In this review, the functional significance of miRNAs in the development and function of the organs of the female reproductive tract is discussed. Initial discussion focuses on the role of miRNAs in the development of the organs of the female reproductive tract highlighting recent studies that clearly demonstrate that mice with disrupted *Dicer1* expression are sterile, fail to develop uterine glands, and have muted estrogen responsiveness. Next, emphasis moves to discussion on our current knowledge on the characterization of miRNA expression in each of the organs of the female reproductive tract. When possible, information is presented and discussed with respect to regulation, function, and/or functional targets of these miRNA within each specific organ of the female reproductive tract.

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Introduction

The female reproductive tract is composed of the ovary, oviduct/fallopian tube, cervix, and uterus. For sustained and successful reproduction, proper development and function of each component is imperative. For normal development and function, a myriad of gene transcription, translation, and posttranslational regulatory mechanisms must be invoked. Unfortunately, our understanding of organogenesis and the molecular mechanisms that regulate the development and differentiation of these tissues lags behind other organ systems. Moreover, recent observations suggest that in addition to transcription, translation, and posttranslational modifications, posttranscriptional gene regulation may play a more pronounced role in cell, tissue, and organ function. In the past several years, micro-RNAs (miRNAs) have been demonstrated to play a novel, yet not thoroughly defined, role in posttranscriptional regulation of gene expression and organ development. The objective of the current review is to summarize our current understanding of those miRNAs that are expressed within the ovary, oviduct, uterus, and cervix. Emphasis is placed upon expression, regulation, and function (where possible) during the course of the menstrual/reproductive cycle.

miRNAs as posttranscriptional regulators

miRNAs are a class of small noncoding regulatory RNAs (18–25 nucleotides) that regulate gene expression posttranscriptionally (Bartel 2004, Vasudevan *et al.* 2007) and are postulated to play a role in normal biological processes while their misexpression has been associated with numerous diseases. miRNA genes primarily reside between genes (intergenic) or within introns (intronic) of genes. Although less common, miRNAs have also been discovered to make up entire introns as well as reside with exons (Berezikov *et al.* 2007, Saini *et al.* 2007). miRNA transcription is most likely mediated by polymerase II or III as the initial long primary RNA transcript (pri-miRNA) is capped (MGpppG) and polyadenylated (Cai *et al.* 2004). miRNA transcription begins with the nucleus where the pri-miRNA transcript binds with the RNA-binding protein DGCR8 (DiGeorge syndrome critical region 8). The pri-miRNA–DGCR8 complex is then cleaved by Drosha (an RNase III enzyme) yielding an ~70 nucleotide stem-loop precursor miRNA (pre-miRNA). The pre-miRNA is then exported from the nucleus via exportin 5 and RAN-GTP into the cytoplasm. Once in the cytoplasm, a second RNase III enzyme, DICER, cleaves the pre-miRNA into a transient RNA duplex of ~18–25 nucleotides. Cleavage of the duplex results in

the generation of two strands with one strand being degraded. The remaining strand is preferentially loaded onto the RNA-induced silencing complex (RISC). The RISC is composed of DICER, a TAR RNA-binding protein (referred to as TRBP) and one of the four different Argonaute (Argo) proteins (Hutvagner & Simard 2008). Once within the RISC, miRNAs bind to the 3'-UTR (or UTR) of the target mRNA transcript. If the binding of the miRNA to the 3'-UTR 'seed sequence' exhibits perfect base-pairing, the mRNA transcript is degraded and mRNA translation does not occur (Jackson & Standart 2007). If bp binding homology between miRNA and 3'-UTR of the mRNA is imperfect, mRNA translation is inhibited. This most likely occurs via an inhibition of translation after initiation (Jackson & Standart 2007) or through an RISC interaction with eukaryotic translation initiation factor 6 (EIF6) preventing assembly of 80S ribosomes (Chendrimada *et al.* 2007). While the majority of the literature supports the notion that miRNAs inhibit translation, there is some evidence that miRNAs can actually enhance translation through alterations in the Argo component of the RISC (Vasudevan *et al.* 2007). Thus, while miRNAs appear to primarily regulate translation in an inhibitory manner, they may also enhance translation in certain biological scenarios.

Functional role of miRNAs during the development of the female reproductive tract

Much like other organ systems (Harfe *et al.* 2005, Harris *et al.* 2006, O'Rourke *et al.* 2007), miRNAs/small RNAs have been shown to be essential for proper development and function of the female reproductive tract. Using a mouse mutant with a hypomorphic *Dicer1* allele, Otsuka *et al.* (2008) were the first to demonstrate that *Dicer1* deficiency resulted in female infertility. In this study, loss of *Dicer1* expression using gene-trap technology resulted in luteal insufficiency and inability to support pregnancy. Associated with these deficiencies was a lack of *miR-17-5p* and *let7b*, which are putative regulators of the antiangiogenic factor TIMP1.

Subsequent studies have also demonstrated reproductive tract abnormalities (such as oviductal cysts, shortened uterine horns and altered oocyte/embryo development) and infertility in female mice in which *Dicer1* was inactivated in Müllerian duct mesenchyme-derived tissues of the reproductive tract using an *Amhr2-Cre* allele (Nagaraja *et al.* 2008). Similarly, Hong *et al.* (2008) observed similar oviduct and uterine defects but no significant impact of *Dicer1* deletion on ovulation rates or embryo development *in vitro*. However, embryos collected from day 3 of pregnancy *in vivo* were developmentally delayed in *Dicer1*-deficient mice compared with wild-type counterparts. Gonzalez & Behringer (2009) also observed similar defects within

the oviduct, degenerated/unfertilized oocytes within the oviductal cysts and an inability to establish pregnancy. Further, histological analysis demonstrated that *Dicer1*-deficient uteri contained less glandular tissue and exhibited what appeared to be early stage adenomyosis. Most recently, Lei *et al.* (2010) explored the role of miRNAs in mouse ovarian development using mice in which *Dicer1* was deleted specifically in follicular granulosa cells. Conditional inactivation of *Dicer1* in the follicular granulosa cells led to an increased endowment of the primordial follicle pool, accelerated early follicle recruitment, and exhibited more degenerate follicles. Taken together, the infertility and reproductive tract abnormalities characteristic of *Dicer1*-deficient female mice strongly suggest that *Dicer1* function and miRNA-mediated posttranscriptional gene regulation are essential for normal female reproductive tract development and function. In the following paragraphs, a presentation of our current knowledge on miRNA expression in each component of the female reproductive tract is presented. When possible, the biological function(s) regulated by these miRNAs and their posttranscriptional target(s) that impact these processes are discussed.

miRNA profiles, regulation, and function in the female reproductive tract

Our understanding of the expression, regulation, and function of specific miRNAs in the organs of the female reproductive tract is just beginning to emerge. The first report, by Liang *et al.* (2007), examined global profiles of distribution and expression of 345 unique miRNAs in 40 normal human tissues obtained from commercial sources. Table 1 displays the most abundant miRNAs (C_t value of 23 or less) detected in the ovary, fallopian tube, uterus, and cervix detected in their study. Notably, all organs expressed abundant levels of *miR-26a*, which has been shown to suppress cell proliferation and tumor growth (Lu *et al.* 2011). *miR-26b* is also expressed in high levels in fallopian tube, uterus, and cervix but not in the ovary and is proposed to regulate apoptosis (Liu *et al.* 2011). *miR-26a* expression was also noted to be abundant in bovine thymus, lymph nodes,

Table 1 Most abundant micro-RNAs (miRNAs) in organs of the human female reproductive tract.

Organ			
Ovary	Fallopian tube	Uterus	Cervix
<i>miR-26a</i>	<i>miR-26a</i>	<i>miR-26a</i>	<i>miR-26a</i>
<i>miR-125b</i>	<i>miR-26b</i>	<i>miR-26b</i>	<i>miR-26b</i>
<i>let7c</i>	<i>miR-21</i>	<i>miR-125b</i>	<i>miR-29a</i>
		<i>miR-16</i>	<i>miR-21</i>
		<i>let7c</i>	<i>miR-23b</i>
			<i>miR-24</i>
			<i>miR-99a</i>

small intestines, and embryo (Coutinho *et al.* 2007). From a functional standpoint, *miR-26a* over-expression is associated with myogenesis, and this is postulated to occur via posttranscriptional repression of Enhancer of Zeste homolog 2 (EZH2; Wong & Tellam 2008). Subsequent studies have revealed that *miR-26a* suppresses tumor proliferation by targeting PTEN (Huse *et al.* 2009) and itself is repressed by MYC (Sander *et al.* 2008). Along these lines, Zhang *et al.* (2011) recently demonstrated that downregulation of *miR-26a* antagonizes apoptosis and facilitates carcinogenesis via the regulation of EZH2 and MTDH. Whether *miR-26a* plays a similar role in the tissue/cells of the female reproductive tract yet remains to be determined.

Since the initial reports that characterized the expression of miRNAs in the reproductive organs of the female tract, numerous studies have been conducted to further define the miRNA profiles as well as their regulation and/or function. In the following, miRNA expression, regulation, function, and identified targets of these miRNAs, whereby these cellular processes are mediated, are discussed.

Ovary

The ovary is essential not only for the production of steroid hormones that prepare the uterus for pregnancy and support pregnancy but also to provide the female gamete that, when fertilized, will develop into a newborn. Within the context of ovarian biology, expression of ovarian miRNAs has been assessed in humans as well as rodents with primary emphasis on the period of follicle growth and ovulation. Choi *et al.* (2007) profiled miRNA expression in the ovaries of newborn mice and found *miR-26a* and *let7c* (both of which were identified in human ovarian tissue (Liang *et al.* 2007) to be among the most abundant miRNAs in this tissue. Further, *miR-709*, which has been identified as a novel murine miRNA for which there is no human homolog, was the most abundant of all miRNAs detected in this study. In a subsequent study, Ro *et al.* (2007) cloned 122 miRNAs from the ovaries of 2-week-old and adult mice and further identified 15 novel miRNAs not reported by Choi *et al.* (2007). Most recently, Ahn *et al.* (2010) performed massive parallel sequencing and identified four novel miRNAs in addition to demonstrating that the *let7* family members and *miR-26a* (*miR-26a-1*) were among the most abundant miRNAs expressed in newborn mouse ovarian tissue.

Functional roles for specific miRNAs within the development of the ovary are just beginning to emerge. *miR-503*, which is highly abundant in mouse ovary, was shown to be markedly downregulated in the ovaries of *Dicer1* null mice (Lei *et al.* 2010). Using a gonadotropin-induced follicular development regime, they noticed in wild-type mice that *miR-503* was downregulated during early follicular development but increased during later

stages, just before ovulation. However, these levels were only about one-half of those in unstimulated mouse ovaries. During luteinization and development of the corpus luteum, *miR-503* levels further decline (about 1/10 of unstimulated ovary levels) to nondetectable levels in mature corpus lutea. Upregulation of *miR-503* in granulosa cells demonstrated that this miRNA downregulated the expression of many target (*Acvr2a* (*ActR1la*), *Acvr2b* (*ActR1lb*), *Fshr*, *Bcl2*, and *Ccnd2*) and nontarget (*Inha*, *Inhba*, *Inhbb*, *Cyp19a1* (*Cyp19a*), *Lhcgr*, *Esr2*, *Ar*, *Cdkn1b*, and *Casp3*) genes associated with granulosa cell proliferation and luteinization.

In the mouse, miRNA-mediated posttranscriptional regulation is required for embryonic germ cell development (Hayashi *et al.* 2008), but the role of individual miRNAs in their development remains unclear. miRNAs from the *miR-290* cluster and the *miR-290–295* clusters are the most abundant miRNAs in mouse embryonic stem cells (Calabrese *et al.* 2007), and they are the first embryonic miRNAs upregulated in the zygote (Tang *et al.* 2007). As this information implies that the *miR-290–295* clusters may be vital to early development of mouse, Medeiros *et al.* (2011) generated *miR-290–295* mutant mice by targeted disruption in embryonic stem cells. While disruption of *miR-290–295* resulted in partial penetrant lethality of mutant embryos, it was also demonstrated that surviving mutant embryos produced female offspring that were infertile, while males showed no such defects. Further analysis revealed that the female infertility was due to premature ovarian failure. Thus, it appears that the *miR-290–295* clusters are essential for proper ovary and follicle development.

miRNA expression profiling of mouse embryonic gonads (Takada *et al.* 2009) identified miRNAs that were expressed in a sex-dependent manner identifying *miR-29b* as female-enriched miRNA. Within the ovaries, *miR-29b* increased from embryonic days 13.5–17.5 and was localized to primordial germ cells. Using luciferase reporter assays, Takada *et al.* (2009) also demonstrated that *miR-29b* targets the DNA methylation enzymes, DNMT3A and DNMT3B. These data may suggest that *miR-29* represses expression of these enzymes allowing in female germ cells to allow escape from male-type methylation of the genome and allow proper development of the ovaries.

While the majority of these studies have focused on mouse ovary, Mishima *et al.* (2008) assessed miRNA expression in adult ovarian tissue and identified 159 miRNA genes with *miR-125b* being one of the most abundant miRNAs expressed. To this point, *miR-26a* appears to be one of the most abundantly expressed miRNAs in both human and newborn mouse ovaries. However, to date, the localization, regulation, and function of this miRNA in ovarian function remain unknown.

More recently, Childs *et al.* (2012) examined the impact of *LET7* family members on ovary development

in the humans. The RNA binding protein, LIN28a (LIN28) is known to target the *LET7* family of miRNAs and its expression decreases at the time during which sex determination occurs and immediately before entry into meiosis (West *et al.* 2009) in the female. The LIN28a targets pri-*LET7a/f/d* and pri-*LET7g* miRNAs decreased between 8- to 11-week gestational age (wga) and 14–16 wga, mirroring the changes in *LIN28a* expression during the process of germ cell differentiation. Thus, it appears that the *LET7* family of miRNAs may be important in the developing ovary.

In addition to focusing on whole ovarian tissue expression profiles, research has also focused more on miRNA expression and function within specific ovarian cell types that play a role in ovarian function (Tables 2 and 3). One of the first reports using mouse mural granulosa cells (Fiedler *et al.* 2008) revealed that *in vivo* 196 and 206 detectable miRNA transcripts were present at 0 and 4 h, respectively, following *in vivo* human chorionic gonadotropin (hCG) administration. Further analysis revealed that hCG significantly increased the expression of 13 miRNAs (4 vs 0 h). Of these, *miR-132* and *miR-212* as well as *miR-21* were the most significantly upregulated. Further studies using isolated granulosa cell culture experiments verified this pattern of expression for *miR-132* and *miR-212*. To determine whether either of these miRNAs impact granulosa estrogen and/or progesterone production, *miR-132* and *miR-212* expression was knocked down using LNA molecules. Knockdown of *miR-132* and *miR-212* alone or in combination had no effect on granulosa steroid production.

A subsequent study by this same group (Carletti *et al.* 2010) demonstrated that *miR-21* plays an antiapoptotic role in ovarian granulosa cells. Knockdown of *miR-21* in granulosa cells *in vitro* as well as *in vivo* knockdown of whole ovarian *miR-21* expression resulted in increased apoptosis. Further, the *in vivo* knockdown of *miR-21* was associated with a reduced ovulation rate. Although *miR-21* was proposed to mediate granulosa cell/ovarian tissue apoptosis, the authors could not demonstrate an alteration in the levels of protein expression of the *miR-21* apoptosis-regulating targets programmed cell death, PTEN, tropomyosin-1, and sprouty homolog 2, suggesting that *miR-21* regulation of apoptosis within the ovary may occur via yet unidentified *miR-21* targets.

miRNAs that control ovarian cell apoptosis and proliferation have also been examined in humans (Sirotkin *et al.* 2010). Ovarian granulosa cells were transfected with different constructs encoding pre-miRNAs, and proliferation and apoptosis were assessed by immunohistochemical localization of PCNA and BAX respectively. Eleven miRNAs stimulated while 53 inhibited expression of PCNA (Table 2). Further, 11 miRNAs stimulated and 46 reduced BAX expression (Table 2). More specifically, knockdown of *miR-15a* expression resulted in enhanced PCNA expression, suggesting that this miR regulates cell proliferation,

while knockdown of *miR-105* and *miR-182* reduces the expression of apoptotic markers, suggesting that these miRNAs stimulate apoptosis. Unfortunately, these studies neither explored the targets of these miRNAs, which may be regulated to modulate cell apoptosis/proliferation, nor did they examine what factors may modulate the expression of these miRNAs.

Sirotkin *et al.* (2009) used an identical approach to identify miRNAs that regulate ovarian cell steroidogenesis. Thirty-six miRNAs that inhibited progesterone release and ten miRNAs that enhanced progesterone release were identified (Table 2). Transfection of granulosa cells with anti-miRs for *miR-15a* and *miR-188* increased progesterone release, suggesting that these two miRNAs were capable of reducing progesterone release. Testosterone release was also affected by miRNAs with 57 miRNAs inhibiting testosterone release, while only one, *miR-107*, stimulating the release of this steroid (20% above control values; Table 2). Lastly, 51 miRNAs inhibited estradiol (E₂) release, while no miRNAs were identified that stimulated E₂ release (Table 2). Although these studies provide the initial identity of miRNAs, which may regulate ovarian granulosa cell steroid release, the mechanisms of action remain unknown.

More recently, two independent studies evaluated miRNA regulation and function using rodent granulosa cells. FSH regulation of miRNA expression on progesterone production using rat granulosa cells was evaluated *in vitro*. Granulosa cells were exposed to FSH and 17 miRNAs were identified as upregulated while 14 miRNAs were determined to be downregulated (Yao *et al.* 2010a). Of these, *miR-23b* increased 1.32-fold, while *miR-29a* and *miR-30d* decreased (0.48- and 0.60-fold change respectively) at 12 h after FSH exposure. In contrast, both *miR-23b* and *miR-30d* expression increased at 48 h post-FSH administration. Bioinformatic analysis was used to predict putative targets of *miR-29a* and *miR-30d*. COL4A1 and BMF (*miR-29a* targets) as well as RNF2 and EED (targets of *miR-30d*) expression decreased in response to FSH administration, suggesting that the expression of these FSH targets may be modulated by miRNAs. However, the role of these factors in granulosa cells was not examined in this study.

Yao *et al.* (2010b) also used mouse pre-antral granulosa cells to identify TGFβ-1-regulated miRNAs. *miR-125b-5p* was one of the most significantly down-regulated miRNAs while *miR-224* was one of the most significantly upregulated. *miR-224* was identified as a potential mediator of TGFβ-1 granulosa cell proliferation as forced expression of *miR-224* increased proliferation by targeting SMAD4. *miR-224* was also shown to promote E₂ release from granulosa cells and this may involve CYP19A1. Of the studies to date, this appears to be the most informative, providing not only information on the regulation of an ovarian miRNA but also its

Table 2 Ovarian granulosa cell micro-RNAs (miRNAs), their regulation, proposed function and transcriptional targets.

miRNAs	Tissue/cell type	Regulation	Function	References
miR-21	Mouse ovary/ granulosa cells Human granulosa cells	Induced by eCG and hCG ^a ND	Antiapoptotic Inhibit progesterone release <i>in vitro</i>	Carletti <i>et al.</i> (2010) Sirotkin <i>et al.</i> (2009)
let7b, let7c, miR-15a, miR-17-3p, miR-96, miR-92, miR-108, miR-133B, miR-134, miR-135, miR-146, miR-181a, miR-1, miR-19a, miR-20, miR-27a, miR-28, miR-29a, miR-98, miR-125b, miR-126, miR-137, miR-183, miR-31, miR-101, miR-105, miR-107, miR-128, miR-129, miR-132, miR-140, miR-141, miR-142, miR-151, miR-188				
miR-16, miR-24, miR-25, miR-122, miR-145, miR-182, miR-18, miR-125a, miR-147, miR-32, miR-103, miR-143, miR-150, miR-152, miR-153, miR-191	Human granulosa cells	ND	Stimulate progesterone release <i>in vitro</i>	Sirotkin <i>et al.</i> (2009)
let7a, let7b, let7c, miR-16, miR-17-3p, miR-24, miR-25, miR-26a, miR-108, miR-122, miR-124, miR-133B, miR-134, miR-135, miR-145, miR-146, miR-155, miR-182, let7d, let7g, miR-18,	Human granulosa cells	ND	Inhibit testosterone release <i>in vitro</i>	Sirotkin <i>et al.</i> (2009)
miR-19a, miR-20, miR-27a, miR-28, miR-29a, miR-98, miR-100, miR-125a, miR-125b, miR-126, miR-136, miR-137, miR-139, miR-147, miR-148, miR-149, miR-183, miR-184, miR-7, miR-9, miR-10a, miR-21, miR-22, miR-23a, miR-23b, miR-30a-3p, miR-31, miR-32, miR-34a, miR-105, miR-128, miR-129, miR-132, miR-133A, miR-140, miR-141, miR-188				
miR-107	Human granulosa cells	ND	Stimulate testosterone release <i>in vitro</i>	Sirotkin <i>et al.</i> (2009)
miR-15a, miR-24, miR-25, miR-26a, miR-95, miR-96, miR-92, miR-108, miR-122, miR-124, miR-135, miR-144, miR-146, let7d, let7g, miR-1, miR-18, miR-19a, miR-20, miR-27a, miR-28, miR-29a, miR-98, miR-125a, miR-125b, miR-126, miR-137, miR-139, miR-148, miR-149, miR-184, miR-7, miR-10a, miR-22, miR-30a-3p, miR-31, miR-32, miR-34a, miR-101, miR-103, miR-105, miR-128, miR-129, miR-132, miR-133A, miR-140, miR-150, miR-151, miR-152, miR-187, miR-188				
miR-108, miR-7, miR-9, miR-105, miR-128, miR-132, miR-141, miR-142, miR-152, miR-188, miR-191	Human granulosa cells	ND	Increase PCNA (cell proliferation)	Sirotkin <i>et al.</i> (2010)
let7b, let7c, miR-15a, miR-16, miR-24, miR-25, miR-26a, miR-96, miR-122, miR-124, miR-133b, miR-134, miR-144, miR-145, miR-155, miR-181a, miR-182, let7d, let7g, miR-1, miR-18, miR-19a, miR-20, miR-27a, miR-28, miR-29a, miR-98, miR-125a, miR-125b, miR-126, miR-137, miR-139, miR-148, miR-149, miR-184, miR-7, miR-10a, miR-22, miR-30a-3p, miR-31, miR-32, miR-34a, miR-101, miR-103, miR-105, miR-128, miR-129, miR-132, miR-133A, miR-140, miR-150, miR-151, miR-152, miR-187, miR-188	Human granulosa cells	ND	Decrease PCNA (cell proliferation)	Sirotkin <i>et al.</i> (2010)
miR-136, miR-137, miR-139, miR-147, miR-148, miR-149, miR-183, miR-10a, miR-21, miR-22, miR-23a, miR-30a-3p, miR-31, miR-32, miR-34a, miR-129, miR-140, miR-143, miR-150, miR-153, miR-187				
miR-15a, miR-96, miR-92, miR-124, miR-18, miR-29a, miR-125a, miR-136, miR-147, miR-183, miR-32	Human granulosa cells	ND	Increase Bax (apoptosis)	Sirotkin <i>et al.</i> (2010)
let7b, let7c, miR-17-3p, miR-24, miR-25, miR-26a, miR-95, miR-133b, miR-134, miR-135, miR-144, miR-145	Human granulosa cells	ND	Decrease BAX (apoptosis)	Sirotkin <i>et al.</i> (2010)
miR-155, miR-181a, let7a				
let7g, miR-1, miR-20, miR-125b, miR-126, miR-137, miR-139, miR-148, miR-149, miR-186, miR-21, miR-22, miR-23a, miR-30a-3p, miR-31, miR-34a, miR-105, miR-107, miR-128, miR-129, miR-132, miR-133a, miR-140, miR-14, miR-150, miR-151, miR-152, miR-153, miR-187, miR-190, and miR-191				

ND, factors that regulate miRNA expression were not determined in the study.

^amiR-21 expression was induced *in vivo* by equine chorionic gonadotropin and *in vitro* by human chorionic gonadotropin (hCG).

potential function and the targets through which it mediates these affects.

In addition to granulosa/follicular cells, substantial information exists on miRNA expression and potential function in ovarian surface epithelial (OSE) cells. The majority of these data are obtained from the comparisons between normal ovarian surface epithelium (NOSE) and cancerous OSE cells. Within the context of normal ovarian physiology, NOSE cells play a pivotal role in repair of the ovarian surface as a result of the ovulatory process. Early interest in the physiology and function of NOSE arose as it became apparent that these cells participate in ovulation and the tissue remodeling that occurs from this. However, with the discovery that the majority of ovarian cancers arise from the NOSE cells (Godwin *et al.* 1993) and subsequent miRNA profiling in ovarian cancer (Iorio *et al.* 2007), these cells became the topic of much more thorough investigations for assessing miRNA function. Table 3 summarizes miRNA expression in ovarian NOSE compared with ovarian cancer tumor

tissue and or cells, their validated targets, and proposed functional pathway, which they regulate.

Based upon the fact that ovarian cancer is a disease characterized by cell invasion, migration, and proliferation, it is not surprising that the bulk of studies assessing miRNA function have focused on miRNAs, which may regulate these pathways (Table 3). Some of the first studies demonstrated that the expression of the *miR-200* family was correlated with the epithelial/mesenchymal phenotype of ovarian cancer cell lines (Park *et al.* 2008). This study as well as other studies (Bendoraitis *et al.* 2010) demonstrated that *miR-200a/b/c*, *miR-141*, and *miR-429* determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressor proteins ZEB1 and ZEB2 as well as regulating oxidative stress via p38 α (Mateescu *et al.* 2011).

Over-expression of miRNAs in ovarian cancer tissue/cells has also been associated with an inflammatory phenotype (Chen *et al.* 2008) and increased cell proliferation, invasion, and migration (Lou *et al.* 2010, Zhang *et al.* 2012a, 2012b). Over-expression

Table 3 Ovarian surface epithelial micro-RNAs (miRNAs), their expression level, proposed function, and transcriptional targets.

miRNAs	Cell/tissue type	↑/↓	Function	Proposed target	Validation ^a	References
<i>miR-200a/b/c</i> , <i>miR-141</i>	NCI60 cell lines ^b 'Epithelial phenotype'	↑	MET	ZEB1, ZEB2	Yes	Park <i>et al.</i> (2008)
	'Mesenchymal phenotype'	↓	EMT			
<i>miR-200a/b/c</i>	HOSE cells, tumor tissue and EOC cell lines ^c	↑	MET	ZEB1, ZEB2	Yes	Bendoraitis <i>et al.</i> (2010)
<i>miR-141</i> , <i>miR-429</i> <i>miR-200a/b/c</i>	Ovarian tumor tissue EOC cell lines ^d	↑	Oxidative stress	p38 α	Yes	Mateescu <i>et al.</i> (2011)
<i>miR-141</i> , <i>miR-429</i> <i>miR-199a</i>	Primary EOC cells, EOC cell lines ^e	↑	Inflammation	IKK β	Yes	Chen <i>et al.</i> (2008)
<i>miR-9</i>	Ovarian cancer cell line ES-2	↓	Cell proliferation	NF- κ B1	Yes	Guo <i>et al.</i> (2009)
<i>miR-15a</i>	Ovarian cancer tissue and ovarian cancer cell lines ^f	↓	Cell proliferation, clonal growth	Bmi1	Yes	Bhattacharya <i>et al.</i> (2009)
<i>miR-16</i>		↓				
<i>miR-34a/b*/c</i>	Ovarian cancer tissue OSE cells ^g	↓	Cell proliferation, invasion, motility	MET	No	Corney <i>et al.</i> (2010)
<i>miR-22</i>	EOC cell lines ^h	↓	Cell migration, invasion	ESR1 ⁱ	No	Li <i>et al.</i> (2010a, 2010b)
<i>miR-21</i>	Ovarian cancer tissue EOC cell lines ^j	↑	Cell proliferation, invasion, migration	PTEN	No	Lou <i>et al.</i> (2010)
<i>miR-25</i>	EOC cell lines	↑	Cell proliferation	Bim	Yes	Zhang <i>et al.</i> (2012a, 2012b)
<i>miR-152</i>		↓	Cell proliferation	Not determined	No	Zhou <i>et al.</i> (2012)
<i>miR-148a</i>						

MET, mesenchymal-to-epithelial transition/reduced aggressiveness; EMT, epithelial-to-mesenchymal transition/increased aggressiveness.

^aTarget validation was performed by 3'-UTR reporter construct assays and/or miR transfection and western analysis. ^bNCI60 cell lines; see Liu. *et al.* mRNA and micro-RNA expression profiles of the NCI-60 integrated with drug activities. *Molecular Cancer Therapeutics* 9 1080–1091 (2010) for list of cell lines. ^cPrimary human ovarian surface epithelial (HOSE) cells, ovarian epithelial tumors, and ovarian cancer cell lines 2008, A1847, A2780, IGROV-1, OVCAR5, OVCAR10, PEO-1, A1847, CAOV3, ES-2, SKOV3, HEY, HAM, OVCAR3, OV90, TOV-21G, TOV-112D, and MCD8105 were studied. ^dCell lines studied were the EOC cell line SKOV3 as well as the mouse fibroblast cells, embryonic kidney cell line 293T, and human breast cancer cell line MDA-MD-468. ^eCell lines studied were cell lines SKOV3, A2780, and CP70. ^fCell lines studied were OVCAR-5, OV-167, CP-70, A2780, and OV-202. ^gmiRNA expression was compared between EOC tumor tissue samples vs that in six normal ovarian surface epithelial primary cell samples. ^hHigh-metastatic human serous ovarian cancer cell SKOV-3ip and HO-8910PM were compared with respective relative low-metastatic human serous ovarian cell lines SKOV-3 and HO-8910. ⁱESR1 was a projected target whose expression was not assessed or validated. ^jEpithelial ovarian cancer tissue, tissue from benign ovarian epithelial cysts, and normal ovarian tissue sections were used as well as OVCAR3 cells.

of *miR-199a* was reported in ovarian cancer cell lines by [Chen et al. \(2008\)](#) who further demonstrated that IKK β expression was reduced in these cells and correlated with pathways associated with inflammation. Over-expression of *miR-21* and *miR-25* was independently reported by [Lou et al. \(2010\)](#) and [Zhang et al. \(2012a, 2012b\)](#), respectively, in ovarian cancer cell lines. Over-expression of *miR-21* ([Lou et al. 2010](#)) was associated with reduced PTEN expression levels; however, it was not determined whether this was a direct or indirect effect of *miR-21* as functional validation was not performed. Over-expression of *miR-25* ([Zhang et al. 2012a, 2012b](#)) was associated with increased cell proliferation, which was mediated via the *miR-25* target Bim.

In addition to increased cell proliferation, migration, and invasion, which result from over-expression of specific miRNAs, there is also strong evidence that under-expression of specific miRNAs also leads to an increase in these cellular events. *miR-9* regulates cell proliferation in ES-2 cancer cells by targeting NF- κ B1 ([Guo et al. 2009](#)) while *miR-15a* and *miR-16* were shown to regulate cell proliferation and clonal growth via Bmi1 ([Bhattacharya et al. 2009](#)). Functional validation studies confirmed that the impact of these miRNAs was directly on their respective targets. In addition to these miRNAs, *miR-22* ([Li et al. 2010a, 2010b](#)) and *miR-152* (as well as *miR-148*, but significantly; [Zhou et al. 2012](#)) have been shown to be suppressed in ovarian cancer cell lines, and this reduction was associated with cell migration and invasion (*miR-22*) as well as cell proliferation (*miR-152*). Li et al. speculated on the *miR-22* targets that may be involved in the proliferative pathway but failed to validate ESR1 as a bonafide target. As such, although the data from these two studies clearly indicate that reduced expression of *miR-22* and *miR-152* is, respectively, associated with enhanced cell migration and invasion as well as proliferation, their targets and whether this is a direct or indirect effect remain to be determined.

In summary, within the context of ovarian granulosa cells, miRNAs have been identified within the ovary, which are under regulation of FSH/LH and appear to contribute to cell proliferation and/or apoptosis as well as steroid production/release. miRNA expression in ovarian surface epithelium has been primarily derived from studies using cancer specimens/cell lines. As such, our current knowledge on these miRNAs primarily focuses on those events conducive to cancer progression including cellular proliferation, invasion, and migration. The reader is encouraged to keep in mind that much of the data with regard to expression and function of miRNAs in OSE cells is compounded by the fact that there is a great deal of variability in the experimental designs. Some studies compare whole tissues (disease vs control) while some studies compare whole tissue vs

isolated cells or cell lines. When assessing the data, one must carefully take into account the models used in these studies. Ideally, consistency should be maintained throughout for more accurate comparison among the study findings. Future research efforts should focus on deciphering the precise mechanisms by which miRNAs regulate these cellular processes within the ovary and whether alterations in the expression of these miRNAs and/or the pathways, which they regulate, are associated with ovarian insufficiencies with respect to ovulation, steroid production, cell differentiation, and infertility.

Oviduct

Despite the marked alteration in oviduct anatomy and function, which results from loss of Dicer function/miRNA expression ([Hong et al. 2008](#), [Nagaraja et al. 2008](#), [Gonzalez & Behringer 2009](#)), very little is known with respect to miRNA expression, regulation, and function within the oviduct. [Nagaraja et al. \(2008\)](#) performed deep sequencing of small RNAs in oviductal tissue from wild-type and *Dicer1* conditional knockout mice and found that 28 miRNAs that were down-regulated in knockout oviducts compared with wild type. mRNA targets of these miRNAs were predicted using TargetScan, PicTar, and miRanda algorithms. The majority of these miRNAs (23/28) were predicted to target genes that were upregulated and included *Wnt5a*, *Cald1*, *Hoxa9*, *Hoxa10*, *Des*, *Apc (Min)*, and *Tagln*, factors involved in mesenchyme-derived structures and Müllerian duct differentiation. Based upon the inverse pattern of expression between these genes and their putative miRNA, it may be suggested that a *Dicer1*-miRNA network may play a role in normal oviduct development and function.

In addition to this study, there is also limited information on miRNA expression, regulation, and function in human oviduct tissue/fallopian tube. [Lee et al. \(2009\)](#) used normal fallopian tube samples as control tissues for assessing miRNA expression in *BRCA1/2* mutation-carrying and nonmutation-carrying high-grade serous ovarian carcinomas. Compared with fallopian tube tissue, 19 miRNAs were significantly upregulated while nine miRNAs were significantly downregulated. Of these, five were further confirmed by qRT-PCR and verified that, compared with fallopian tubes, *miR-34a*, *miR-143*, and *miR-145* were significantly lower in the ovarian cancer specimens, while *miR-29a* and *miR-29b* were significantly higher. While none of these miRNAs were considered among the most abundantly expressed miRNAs in fallopian tube/oviduct tissue ([Liang et al. 2007](#)), all these miRNAs were detected in the study by [Liang et al. \(2007\)](#); *miR-29a* > *miR-145* > *miR-143* > *miR-29b* > *miR-34a*.

While the study by [Lee et al. \(2009\)](#) did not address the potential functional significance of these miRNAs,

one may speculate that based upon low expression in carcinoma tissue and the proposed targets (as determined by TargetScan, PicTar, and miRanda algorithms), that *miR-34a*, *miR-143*, and/or *miR-145* may regulate pathways associated with cellular proliferation (Akao *et al.* 2010). Similarly, *miR-29a* and *miR-29b*, which were elevated in ovarian carcinomas, may regulate collagen-dependent extracellular matrix homeostasis. Li *et al.* (2009) have demonstrated that *miR-29* suppresses the expression of several collagens. These observations, coupled with the fact that *miR-29* increases MMP2/MMP9 expression via decreased DNA methylation (Chen *et al.* 2011), point toward a potential role of *miR-29* in enhanced cell migration.

In summary, aside from these few studies, there is currently a paucity of information with respect to miRNA expression and, more so, function within the fallopian tube. Information gathered from animal models in which *Dicer* expression is deleted (Hong *et al.* 2008, Nagaraja *et al.* 2008, Gonzalez & Behringer 2009) and miRNA expression is reduced (Nagaraja *et al.* 2008) strongly demonstrates a significant impact on oviductal phenotype and infertility. If we extrapolate data from other systems and take into account these potential functions, one may infer that *miR-29a*, *miR-29b*, *miR-143*, and *miR-145* may regulate key pathways for the normal development and function of the oviducts. However, to be able to make such conclusions will require examination of these posttranscriptional regulators in this organ in more detail.

Uterus: endometrium

The majority of our information on uterine miRNAs has primarily been derived from studies that have compared miRNA expression between women without

endometriosis ('normal' controls) to that of women with endometriosis. The intent of the discussion on uterine miRNAs is to outline miRNAs that have been identified in 'normal' uterine tissue. For those readers interested in miRNA expression between eutopic and ectopic/endometriotic tissue and the potential function of these miRNAs in the pathogenesis of endometriosis, there are several recent publications and reviews (Ohlsson Teague *et al.* 2009, Teague *et al.* 2010, Ramón *et al.* 2011) on this topic.

The first assessment of endometrial miRNA expression was by Pan *et al.* (2007) in which they examined endometrial tissue from 'normal' women and compared that with endometrium from women with endometriosis to establish human endometrial miRNA profiles. Sixty-five miRNAs were detected above a pre-determined threshold level. In endometrium from 'normal' women (women without endometriosis in this study), *miR-125b*, *miR-21*, *miR-145*, *miR-26a*, *miR-23b*, *miR-29a*, and *miR-99a* were among the most abundant. In endometrium from women with endometriosis as well as endometriotic tissue, there was a significant reduction in the expression of all these miRNAs as well as several other miRNAs. miRNA expression in normal endometrium was further assessed based upon cell type, focusing on endometrial stromal cells and glandular epithelial cells. Thirty-two miRNAs were shown to be differentially expressed between cell types. Of these, the investigators focused on examining the steroidal regulation of *miR-20a*, *miR-21*, and *miR-26a*. E₂ decreased stromal cell expression of *miR-20a* and *miR-21* but increased expression of *miR-26a*, while medroxyprogesterone acetate significantly decreased expression of all the three miRNAs (Table 4). In glandular epithelial cells, E₂ decreased expression of *miR-20a* and *miR-21* but increased expression of *miR-26a*, while

Table 4 Uterine cell micro-RNAs (miRNAs), their regulation, proposed function, and transcriptional targets.

miRNAs	Cell/tissue type	Regulation	References
<i>miR-20a</i> <i>miR-21</i> <i>miR-26a</i>	Human stromal cells (<i>in vitro</i>)	E ₂ decreased/MPA decreased E ₂ decreased/MPA decreased E ₂ increased/MPA decreased	Pan <i>et al.</i> (2007)
<i>miR-20a</i> <i>miR-21</i> <i>miR-26a</i>	Human glandular epithelial cells (<i>in vitro</i>)	E ₂ decreased/MPA increased E ₂ decreased/MPA decreased E ₂ increased/MPA increased	Pan <i>et al.</i> (2007)
<i>miR-17-5p</i> <i>miR-542-3p</i> <i>miR-23a</i> <i>miR-23b</i>	Human stromal cells (<i>in vitro</i>)	E ₂ increased/MPA increased E ₂ increased/MPA increased E ₂ decreased/MPA decreased E ₂ decreased/MPA no affect	Toloubeydokhti <i>et al.</i> (2008)
<i>miR-17-5p</i> <i>miR-542-3p</i> <i>miR-23a</i> <i>miR-23b</i>	Human glandular epithelial cells (<i>in vitro</i>)	E ₂ increased/MPA increased E ₂ decreased/MPA decreased E ₂ decreased/MPA increased E ₂ increased/MPA increased	Toloubeydokhti <i>et al.</i> (2008)
<i>miR-155</i> <i>miR-429</i> <i>miR-451</i>	Mouse uterus (<i>in vivo</i>)	E ₂ increased	Nothnick and Healy (2010)
<i>miR-181b</i> <i>miR-204</i>	Mouse uterus (<i>in vivo</i>)	E ₂ decreased	Nothnick & Healy (2010)

E₂, 17β estradiol; MPA, medroxyprogesterone acetate.

medroxyprogesterone acetate increased *miR-20a* and *miR-26a* but decreased *miR-21* expression (Table 4).

In a subsequent study by this same group (Toloubeydokhti *et al.* 2008), *miR-17-5p*, *miR-23a*, *miR-23b*, and *miR-542-3p* expression was assessed, and it was demonstrated that *miR-23b* and *miR-542-3p* were expressed in lower levels and that *miR-17-5p* was expressed at higher levels in paired eutopic and ectopic endometrial tissue compared with 'normal' eutopic endometrium. In isolated stromal cells, E₂ increased the expression of *miR-17-5p* and *miR-542-3p* but decreased the expression of *miR-23a* and *miR-23b* (Table 4). MPA increased expression of *miR-17-5p* and *miR-542-3p*, decreased the expression of *miR-23a*, and had no effect on *miR-23b* expression (Table 4). In isolated glandular epithelial cells, E₂ decreased the expression of *miR-542-3p* and *miR-23a* but increased the expression of *miR-17-5p* and *miR-23b* (Table 4). MPA increased the expression of all miRNAs but *miR-542-3p*, which was significantly decreased (Table 4). In this and the previously cited study (Pan *et al.* 2007), steroidal regulation could be blocked for some of the miRNAs with ICI-182 780 and RU-486 co-treatment (respectively), suggesting a complex mechanism for steroidal regulation of miRNAs in endometrial stromal and glandular epithelial cells.

Burney *et al.* (2009) utilized a similar study design in comparing miRNA expression in early secretory endometrium from women with and without endometriosis. Early secretory endometrium from women with endometriosis expressed significantly lower levels of expression for *miR-34c-3p*, *miR-34c-5p*, *miR-9*, *miR-9**, *miR-34b**, and the unannotated miRPlus_42 780. Although additional miRNA profile information was not provided in this report, one may conclude that these miRNAs are expressed in early secretory endometrium from women without endometriosis and may play a role within the endometrium during the menstrual cycle.

To date, only one study has examined miRNA expression in endometrial tissue/cells from women free of reproductive tract disorders such as endometriosis. Kuokkanen *et al.* (2010) assessed miRNA (and mRNA) expression between endometrial epithelial cells during the late proliferative and midsecretory menstrual phases in hopes of elucidating the action of progesterone in opposing the effects of estrogen on these parameters. Using bioinformatic analysis, the authors discovered that the differentially expressed mRNAs were associated with cell cycle regulation (enriched in late proliferative endometrial epithelium) and WNT signaling (enriched in proliferative phase endometrial epithelium). Twelve miRNAs were significantly upregulated in the midsecretory-phase specimens compared with those from the late proliferative phase and are proposed to target many cell cycle genes. In accordance with the function of miRNAs, *miR-29b*, *miR-29c*, *miR-30b*, *miR-30d*, *miR-31*, *miR-193a-3p*, *miR-203*, *miR-204*, *miR-200c*, *miR-*

210, *miR-582-5p*, and *miR-345* were upregulated while their target genes were decreased. These findings suggest that these miRNAs may regulate protein translation by reducing/inducing decay of cell cycle gene target transcripts and in turn suppress cell proliferation.

In addition to information gleaned from studies that incorporated human endometrial tissue, there is also limited information on miRNA profiles in mouse uterine tissue, with the bulk focusing on identifying miRNAs that are associated with and may play a role in embryo implantation. Initial characterization was performed by Chakrabarty *et al.* (2007) in which expression profiling was performed with oligonucleotide microarrays and compared the expression patterns of 380 miRNAs common to humans, mice, and rats, in day 1 (pre-receptive; estrogen dominance) and day 4 (receptive; progesterone dominance) pregnant mouse uteri (where day 1 represents the presence of a vaginal plug). Thirty-two miRNAs were upregulated during the receptive phase (progesterone dominance) and five were down-regulated compared with the prereceptive (estrogen dominance) phase. Of those upregulated, *miR-101a* and *miR-199a** were further examined and verified to regulate cyclo-oxygenase-2 (COX2) expression, an enzyme that is essential for embryo implantation. Additional studies have suggested that *miR-21* (Hu *et al.* 2008), *let7a* (Xia *et al.* 2010a), and *miR-320* (Xia *et al.* 2010b) may be important for embryo implantation in rodents.

A recent report from my laboratory (Nothnick & Healy 2010) demonstrated by miRNA array and confirmed by qRT-PCR that *miR-155*, *miR-429*, and *miR-451* are upregulated in the mouse uterus by estrogen while *miR-181b* and *miR-204* are downregulated (Table 4). This regulation is mediated via the classical estrogen receptor pathway. Additional unpublished observations from our research group demonstrate that within the mouse uterus from ovariectomized mice, *miR-709* is the most abundant miRNA followed by the *let7* family members and *miR-26a*.

Along the lines of estrogen regulation of uterine miRNAs, a recent study dissected the potential regulation of endometrial miRNAs and the mechanisms by which they regulate cellular proliferation. Zhang *et al.* (2012a, 2012b) demonstrated that estrogen increased estrogen receptor-positive endometrial adenocarcinoma cell proliferation and that this was associated with an upregulation of BCL2 and concurrent downregulation of BAX. As Bax repression was determined to occur posttranscriptionally, miRNA assessment revealed that members of the *let7* family (*let7a-g*) and *miR-27a* targeted BAX transcript. Surprisingly, as discussed above, none of these miRNAs were upregulated in human endometrial cells or mouse uterus in response to estrogen administration.

In addition to those studies that have addressed regulation of uterine miRNA expression, there is also

emerging data on the function of miRNAs within endometrial cells with the bulk of this information derived from studies that have incorporated endometrial carcinoma cell lines. The first description and speculation on the function of miRNAs in endometrial/uterine tissue was presented by Pan *et al.* (2007). These authors proposed that many of the miRNAs expressed within the endometrium may regulate pathways that are essential to normal endometrial biology and, when dysregulated, lead to endometrial pathologies such as endometriosis and endometrial cancer. These pathways include inflammation, apoptosis, proliferation, angiogenesis, and differentiation. Our current understanding on the functional roles of specific miRNAs in endometrial cells is derived primarily from studies incorporating human endometrial adenocarcinoma cell lines as outlined below and summarized in Table 5.

The majority of studies that have focused on determining the specific functional roles of miRNAs in endometrial cells primarily examined cell proliferation. Of the current data, the miR-200 family is the most well studied. Snowdon *et al.* (2011) reported that *miR-200a/b/c*, *miR-141*, and *miR-429* were upregulated in both endometrioid endometrial adenocarcinoma and complex atypical hyperplasia compared with normal control endometrium. Subsequent studies (Lee *et al.* 2011, Park *et al.* 2012) revealed similar studies in cancerous tissues as well as endometrial adenocarcinoma cell lines and further documented a functional role for these miRNAs. Use of anti-miRs for each of these miRNAs suppressed HEC-1A cell proliferation while only anti-*miR-141*, -200c, and -429 inhibitors reduced growth of Ishikawa cells (Lee *et al.* 2011). Park *et al.* (2012) further demonstrated that *miR-200c* could modulate cell proliferation, survival, and apoptosis in HEC-1A and Ishikawa cells. Further differential analysis of mRNA between cells transfected with control or pre-*miR-200c* revealed that *miR-200c* mediation of these

cellular events was in part via downregulation of transcript for the tumor suppressor protein BRD7.

Three additional studies have evaluated the potential role of miRNAs in modulation of tumor suppressor genes in the context of endometrial adenocarcinomas. FOXO1 is also a tumor suppressor that is reduced in endometrial carcinoma (Goto *et al.* 2008, Myatt *et al.* 2010). Using miRNA target prediction programs, Myatt *et al.* (2010) identified a panel of highly conserved miRNAs that could potentially target the 3'-UTR of FOXO1 transcript. Of these, *miR-9*, *miR-27*, *miR-96*, *miR-128*, *miR-153*, *miR-182*, *miR-183*, and *miR-186* upregulation correlated with loss of FOXO1 expression in both endometrial carcinoma tissue and Ishikawa cells. Individual over-expression of all these miRNAs in HEC-1B cells except *miR-128* reduced FOXO1 protein expression and when inhibitors of *miR-9*, *miR-27*, *miR-96*, *miR-153*, *miR-183*, and *miR-186* were transfected as a pool into Ishikawa cells, cell cycle arrest and apoptosis were induced.

FOXC1 belongs to the same family of Forkhead box transcription factors as FOXO1 and its misexpression has also been associated with carcinogenesis (Myatt & Lam 2007). Through analysis of differential expression, endometrial cancer expressed a set of dysregulated miRNAs that included *miR-7*, *miR-149*, *miR-449b*, and *miR-204*. Of these, *miR-204* was shown to target FOXC1 and functionally regulate endometrial adenocarcinoma (HEC-1A) cell migration and invasion (Chung *et al.* 2012). Similarly, the tumor suppressor, TP53INP1, is also modulated by miRNAs and its miRNA-induced suppression is associated with enhanced cell proliferation (Jiang *et al.* 2011). More specifically, *miR-125b* was found to be upregulated in type II endometrial carcinoma cells. Over-expression and inhibition studies using adenocarcinoma cell lines revealed that *miR-125b* modulated *in vitro* and *in vivo* cell proliferation and invasion and that these events were mediated via TP53INP1.

In summary, miRNA profiles have been generated for human and rodent uterine tissues, and steroidal

Table 5 Uterine micro-RNAs (miRNAs), their expression level, proposed function, and transcriptional targets.

miRNAs	Cell/tissue type	↑/↓	Function	Proposed target	Validation ^a	References
<i>miR-200a/b/c</i>	HEC-1A, Ishikawa cell lines	↑	Proliferation	ND	Yes	Lee <i>et al.</i> (2011)
<i>miR-141</i> , <i>miR-429</i> <i>miR-200c</i>	HEC-1A, Ishikawa cell lines	↑	Proliferation	BRD7	Yes	Park <i>et al.</i> (2012)
<i>miR-9</i> , <i>miR-27</i> , <i>miR-96</i> , <i>miR-153</i> , <i>miR-182</i> , <i>miR-183</i> <i>miR-186</i>	Endometrial carcinoma tissue, Ishikawa cells	↑	ND	FOXO1 (decreased)	ND	Myatt <i>et al.</i> (2010)
<i>miR-7</i> , <i>miR-149</i> , <i>miR-449b</i> , <i>miR-204</i> <i>miR-204</i>	HEC-1B cells Endometrial carcinoma tissue HEC-1A cell line	↑	Proliferation ND Migration, invasion	FOXO1 ND FOXC1	Yes ND Yes	Myatt <i>et al.</i> (2010) Myatt & Lam (2007) Myatt & Lam (2007)
<i>miR-125b</i>	Type II endometrial carcinoma cells	↑	Proliferation invasion (<i>in vitro</i> and <i>in vivo</i>)	TP53INP1	Yes	Jiang <i>et al.</i> (2011)

ND, function or proposed target was not determined in the study.

^aTarget validation was performed by 3'-UTR reporter construct assays and/or miR transfection and western analysis.

regulation of some of these miRNAs have been examined in isolated stromal and glandular epithelial systems. Emerging data from studies that have incorporated that human endometrial adenocarcinoma cell lines has shed some insight into the potential function of these miRNAs within the cells of the endometrium and the pathways by which they may do so. The majority of the current information suggests that uterine miRNAs appear to regulate cellular proliferation, migration, and/or invasion and that the members of the *miR-200* family appear to be major players in these processes.

Uterus: myometrium

The myometrium is the muscular wall of the uterus and lies between the internal layer (endometrium) and the outer layer (the perimetrium). The chief function of the myometrium is during the birth process to provide the contractile forces necessary for expulsion of the fetus. During pregnancy, high progesterone levels keep the myometrium/uterus quiescent. [Renthall et al. \(2010\)](#) recently examined the potential role of miRNAs as mediators of hormonally modulated contractile targets in the pregnant uteri of both humans and mouse. Microarray analysis was performed on myometrial tissue from mice before (day 15.5 postcoital) and just before term pregnancy (day 18.5 postcoital with day 19 being term). The *miR-200* family (*miR-200a/b/c*, *miR-141*, and *miR-429*) was upregulated in myometrium from the day 18.5 group compared with the day 15.5 group ([Table 6](#)). Associated with this upregulation was a decrease in the expression of proposed targets, ZEB1 and ZEB2. *miR-200* family and ZEB1/ZEB2 expression was conserved between mouse and human as the *miR-200* family was upregulated and ZEB1 and ZEB2 downregulated in laboring human myometrium compared with myometrium from term, nonlaboring women. Associated with the downregulation of ZEB1/ZEB2 in both mouse and human myometrium was an increase in expression of CXN43 and OXTR. Additional mouse models of preterm labor were used and revealed that there was an upregulation of the *miR-200* family and downregulation of ZEB1/ZEB2.

It was further revealed that ZEB1 is directly upregulated by progesterone and that ZEB1 and ZEB2 inhibit the expression of contractile proteins CXN43 and OXTR. Collectively, these findings suggest that progesterone regulates the expression of the *miR-200* family and their targets ZEB1 and ZEB2 in modulating uterine contractility during pregnancy and labor.

Outside of this single report on miRNAs in myometrial tissue during pregnancy, the majority of our understanding on miRNA expression, function, and regulation in myometrial tissue and/or cells comes from studies focusing on the role of miRNAs in the pathogenesis of uterine fibroids. The first study to assess myometrial miRNA expression was conducted by [Wang et al. \(2007\)](#) where they generated a miRNA signature associated with race, tumor size, and target gene activity. Two hundred and six miRNAs were examined and 45 were found to be dysregulated in uterine leiomyomas. Of these, the five most significantly upregulated miRNAs in leiomyomas were *let7* family, *miR-21*, *miR-23b*, *miR-27a*, and *miR-30a*. *let7* expression was significantly higher in small (<3 cm) leiomyomas compared with large (>10 cm) leiomyomas, and there was an inverse correlation between *let7* expression and its proposed target HMGA2 and validity of target was confirmed by transfection of leiomyoma cells with *let7* ([Table 6](#)). In addition to the upregulated miRNAs, *miR-29b*, *miR-32*, *miR-144*, *miR-197*, and *miR-212* were also shown to be significantly downregulated in leiomyomas. While the predicted targets for all of these miRNAs were reported by [Wang et al. \(2007\)](#), other than *let7*, none of them were validated in their study.

A subsequent study by [Marsh et al. \(2007\)](#) identified 46 miRNAs that were differentially expressed in leiomyomas of which 19 were upregulated and 27 were downregulated. Of these, *miR-542-3p* was the most significantly upregulated (11.8-fold) and *miR-498* was the most significantly downregulated (2.4-fold). While the authors validated the expression of *miR-21*, *miR-34a*, *miR-125b*, *miR-139*, and *miR-323* by qRT-PCR, they did not examine regulation or function of these miRNAs in the pathogenesis of leiomyomas. As *miR-542-3p* has been proposed and validated to target

Table 6 Myometrial micro-RNAs (miRNAs), their expression regulation, proposed function, and transcriptional targets.

miRNAs	Cell/tissue type	↑/↓	Function	Proposed target	Validation ^a	References
<i>miR-200a/b/c</i> <i>miR-141</i> <i>miR-429</i>	Myometrium (human) Myometrium (mouse)	↑ by P ₄	Uterine quiescence	ZEB1/ZEB2	No	Renthall et al. (2010)
<i>miR-200a/b/c</i> <i>miR-141</i> <i>miR-429</i>	UtLM leiomyoma cell line	↓	Cell growth	TUBB, CYP1B1, CTBP2	Yes	Zavadil et al. (2010)
<i>let7</i>	Leiomyoma tissue and cells	ND	Adipogenesis, mesenchymal differentiation	HMGA2	Yes	Wang et al. (2007)

P₄, progesterone; ND, not determined.

^aTarget validation was performed by 3'-UTR reporter construct assays and/or, miR transfection, and western analysis.

survivin and suppress cell growth (Yoon *et al.* 2010), the potential role of this miRNA in the pathogenesis of leiomyomas is unclear at this time. *miR-498* has been proposed to target ZEB2 (based on TargetScan analysis). Thus, reduced levels of *miR-498* in leiomyomas may be associated with increased levels of ZEB2. However, the potential role of ZEB2 in the pathogenesis of leiomyomas has yet to be examined.

Pan *et al.* (2008) evaluated miRNA expression profiles in paired myometrium and leiomyoma tissue, myometrial smooth muscle cells (MSMCs) and leiomyoma smooth muscle cells (LSMC) isolated from these tissues, as well as in the leiomyoma cell lines T-LSMC and SK-LMS-1. They identified 91 miRNAs that were expressed above myometrium thresholds. Of these, *miR-20a*, *miR-21*, *miR-26a*, *miR-18a*, *miR-206*, *miR-181a*, and *miR-142-5p* were confirmed by qRT-PCR and their steroidal regulation assessed in MSMC, LSMC, T-LSMC, and SK-LMS-1. Leiomyomas expressed higher levels of *miR-20a*, *miR-21*, *miR-26a*, and *miR-206* compared with myometrium. *miR-142-5p* expression was lower in leiomyomas compared with myometrium from Caucasians but not in African Americans. African Americans also expressed lower levels of *miR-181a* in leiomyomas compared with matched myometrium while Caucasians expressed higher levels of *miR-181a* in leiomyoma tissue vs myometrium.

When cells isolated from leiomyoma (LSMC) and myometrium tissue (MSMC) were compared with tLSMC and SKLM cell lines, it was revealed that both cell lines expressed significantly higher levels of expression of *miR-20a* and *miR-26a* compared with both MSMC and LSMC, but LSMC expression was lower than that in MSMC. In contrast, *miR-21* expression was lower in LSMC, tLSMC, and SKLM compared with MSMC, but the cell lines exhibited significantly greater levels of *miR-21* expression compared with MSMC.

Examination of the steroidal regulation of these three miRNAs in MSMC and LSMC revealed that estrogen and progesterone modulated miRNA expression in a cell-type and steroid-specific manner. Specifically, estrogen decreased *miR-21* expression in MSMC but not in LSMC. The progesterone analog, MPA, increased *miR-21* expression in LSMC but not in MSMC. Further, both estrogen and MPA increased *miR-26a* expression in MSMC but reduced expression of this miRNA in LSMC. Neither estrogen nor MPA affected *miR-20a* expression in either MSMC or LSMC.

More recently, Zavadil *et al.* (2010) profiled and analyzed the function of miRNAs and their target products in human leiomyoma tissue. Using uterine leiomyomas (> 10 cm) from African American women, predicted gene targets of the five most highly upregulated (*miR-21*, *miR-23b*, *miR-27a*, *miR-30a*, and *let7s*) and downregulated (*miR-29b*, *miR-32*, *miR-144*, *miR-197*, and *miR-212*) miRNAs were determined by miRNA prediction methods (TargetScan and PicTar). There were

249 downregulated putative mRNA targets that corresponded with the five upregulated miRNAs and 97 upregulated putative mRNA targets that corresponded with the five downregulated miRNAs. Of the dysregulated mRNAs, protein localization was assessed for 16 of these (EGFR, ESR1 (ER α), GRIP1, Hamartin, HMGA1, HMGA2, IGF1, IGF2, Ki-67, TYMP (PD-ECGF), PIK3R1 (PI3K), PGR (PR-A), RARA (RAR α), RXRA (RXR α), TGFA, and TSC2). There was an inverse correlation between Ki-67 expression and that of LET7. Moderate negative correlations were found between EGFR and *miR-194-1* as well as between TGF α and *miR-199a-2*. Overall, most gene products and their corresponding miRNAs exhibited negative correlations.

One interesting aspect of the study by Zavadil *et al.* (2010) was the further functional validation of the *miR-200* family and its putative targets. Both *miR-200a* and *miR-200b* were downregulated in leiomyomas while many of their targets were upregulated. To demonstrate validity of a portion of these targets, UtLM cells, which stably over-express *miR-200a*, were evaluated for protein expression and cell growth. Over-expression of *miR-200a* was associated with reduced levels of TUBB, CYP1B1, and CTBP2 and this reduction in protein expression was correlated with growth inhibition (Table 6), suggesting that the reduced level of *miR-200a* may impact cell growth relevant to the pathophysiology of uterine leiomyomas.

In summary, myometrial miRNAs (*miR-200* family) appear to be necessary for normal pregnancy and labor where they regulate uterine contractility. Outside of this report, most of our information on myometrial miRNAs is derived from studies, which have focused on differential profiles between myometrium and leiomyomas (fibroids). Along these lines, current information is primarily limited to assessment of cell growth and proliferation and steroidal regulation of myometrial/leiomyoma miRNAs. As myometrial function is essential to parturition, additional studies are required to enhance our understanding on the regulation and function of miRNAs within this muscle layer. This analysis should also expand into myometrial dysfunction not only associated with the pathogenesis of uterine leiomyomas but also abnormal myometrial function/contraction during abnormal labor/birth.

Cervix

Understanding the biology of the cervix is essential to the understanding of process of normal parturition as well as abnormalities in this process. In an attempt to determine whether cervical dilatation and remodeling after term parturition in the human were associated with changes in miRNA expression, Hassan *et al.* (2010) assessed cervical biopsy specimens from patients who had delivered spontaneously (term; labor) compared with patients who underwent elective cesarean section with

an unripe cervix (term; not in labor). miRNA analysis revealed that 226 miRNAs were expressed in human term cervical tissue. Of these, *miR-223*, *miR-34b*, and *miR-34c* were upregulated in cervical tissue from women who underwent spontaneous labor compared with those who did not. miRBase algorithm was used to predict protein targets of these miRNAs. Using previously published microarray data, genes that may be targeted by these miRNAs whose expression was significantly upregulated were identified and included Claudin-8 (*miR-223* target), arachidonate 12-lipoxygenase (*miR-34b* target), and calmodulin-like-3 (*miR-34c* target).

Most of our understanding on miRNA expression within the cervix has been derived from studies using human cervical cancer specimens and/or cervical cancer cell lines (Lui *et al.* 2007, Lee *et al.* 2008, Martinez *et al.* 2008, Wang *et al.* 2008, Li *et al.* 2010a, 2010b). Lui *et al.* (2007) used cervical carcinoma cell lines (SW756, C4I, C33A, CaSki, SiHa, and ME-180) and normal cervical specimens to generate cervical miRNA profiles. One hundred and sixty-six miRNAs were expressed in cervical cancer cell lines and normal cervical tissue; six miRNAs were differentially expressed between the two cell/tissue types. Two miRNAs in particular (*miR-21* and *miR-143*) were further examined in matched pairs of human cervical cancer and normal cervical samples. Increased expression of *miR-21* and reduced expression of *miR-143* were noted in the cervical cancer specimens, suggesting that these miRNAs may prove useful as tumor markers.

Subsequently, Lee *et al.* (2008) used qRT-PCR to assess miRNA expression in early stage invasive squamous cell carcinomas and normal cervical squamous epithelial specimens. Sixty-eight miRNAs were upregulated and two were downregulated in carcinoma specimens. Two of the most significantly upregulated miRNAs were *miR-127* and *miR-199a*. *miR-127* expression was significantly associated with lymph node metastasis. Using SiHa and ME-180 cancer cell lines transfected with anti-*miR-199a*, the investigators demonstrated that *miR-199a* expression was associated with cell growth and that incorporation of anti-*miR-199a* oligonucleotides with the anticancer agent cisplatin potentiated the suppression of cell growth by this agent.

CaSki-2 cervical cancer cells were used to clone 174 miRNAs of which *miR-21*, *miR-24*, *miR-27a*, and *miR-205* were the most abundant with one novel miRNA (*miR-193c*) also being reported (Wang *et al.* 2008). Ten of the 174 miRNAs were further studied. *miR-143* and *miR-145* expression was undetectable in the cervical cancer cell lines as well as cervical cancer tissue and *miR-126* was also downregulated in the tissue specimens. In contrast, *miR-15b*, *miR-16*, *miR-146a*, and *miR-155* were upregulated in cervical cancer tissue specimens. To assess the potential role of some of

these miRNAs in cervical carcinogenesis, functional studies were conducted. *miR-143* and *miR-145* were shown to suppress cell growth, suggesting that their downregulation/absence in cervical cancer cells/tissue may promote cell proliferation. In contrast, *miR-146a* promoted cell proliferation, suggesting that the detected upregulation of the miRNA in cervical cancer may be growth promoting.

Subsequent studies have assessed the potential impact of miRNA dysregulation in cervical cancer and cell proliferation (Table 7). *miR-21* is perhaps the most well-studied miRNA among cancer biology (Fu *et al.* 2011) and is considered a major regulator of cell proliferation. Using HeLa cells, Yao *et al.* (2009) demonstrated that *miR-21* knockdown suppresses cell proliferation, colony growth, and colony size. Associated with these decreases was an increase in the tumor suppressor PDCD4 protein levels. Luciferase reporter assays were used to validate PDCD4 as a direct target of *miR-21*. While *miR-21* appears to be upregulated in cervical cancers, *miR-100* (Li *et al.* 2011a, 2011b) and *miR-372* (Tian *et al.* 2011) expression is reduced. *miR-100* expression was examined in normal cervical epithelium, cervical intraepithelial neoplasia, and cervical cancer in addition to five cervical cell lines (Li *et al.* 2011a, 2011b). qRT-PCR analysis revealed that *miR-100* was highest in normal cervical epithelium and decrease with increasing stage of cancer. Functional significance of reduced *miR-100* was confirmed by over-expression/knockdown studies, and it was determined that *miR-100* regulated cell proliferation, cell cycle, and apoptosis. Further, these effects of *miR-100* were mediated at least in part through regulation of the mitotic checkpoint protein, PLK1, whose expression is elevated in cervical cancer (Gao *et al.* 2006). In a similar manner, *miR-372* was shown to regulate cell growth and that this involved targeting of CDK2 and cyclin A1. Collectively, these data imply that the upregulated expression of *miR-21* characteristic of cervical cancer most likely enhance cell proliferation via downregulation of PDCD4 while the suppressed levels of *miR-100* and *miR-372* may modulate cell proliferation via PLK1 and CDK2/cyclin A1 respectively.

In addition to alterations in cell proliferation pathways, two recent studies have focused on the pro-apoptotic pathways. P2RX7 (P2X7) is a membrane-bound, ligand-operated channel that mediates apoptosis, which is expressed in normal human ectocervical tissue and human ectocervical-vaginal epithelial cells (hEVECs) but is down regulated in ectocervical cancer and HeLa cells as well as endometrial cancer (Zhou *et al.* 2008). Associated with this reduction in P2RX7 expression is an elevation of *miR-186a* and *miR-150* in HeLa cells compared with hVEC cells. To determine whether these miRNAs play a functional role in the regulation of P2RX7, hVEC, and HeLa cells were transfected with *miR-186* and *miR-150* inhibitors. Both

Table 7 Cervical micro-RNAs (miRNAs), their expression level, proposed function, and transcriptional targets.

miRNAs	Cell/tissue type	↑/↓	Function	Proposed target	Validation ^a	References
<i>miR-199a, miR-143 and miR-145</i>	Cervical cancer tissue	↓		ND	Yes	Yes Lee <i>et al.</i> (2008)
<i>miR-21</i>	SiHa and ME-180 cell lines		Cell growth			
	HeLa cancer cell line	↑	Proliferation, colony growth and size	PDCD4	Yes	Yao <i>et al.</i> (2009)
<i>miR-100</i>	CIN, cervical cancer	↓				
	Cervical cancer cell lines		Proliferation, cell cycle, apoptosis	PLK1	Yes	Li <i>et al.</i> (2011a)
<i>miR-372</i>	Cervical cancer tissue	↓	Cell growth	CDK2, cyclin A1	Yes	Tian <i>et al.</i> (2011)
<i>miR-372</i>	HeLa cells		Pro-apoptotic	P2RX7	Yes	Zhou <i>et al.</i> (2008)
<i>miR-150, miR-186a</i>	hEVEC cells	↓	Antiapoptotic	P2RX7 (miR-150) ^b	Yes	Zhou <i>et al.</i> (2008)
<i>miR-150, miR-186a</i>	HeLa cells	↑	Cell proliferation, antiapoptotic			
<i>miR-886-5p</i>	Cervical cancer tissue	↑				
	H8 cervical cancer cell line			BAX	Yes	Li <i>et al.</i> (2011b)
<i>miR-34a</i>	Cervical cancer tissue cervical, choriocarcinoma cell lines	↓				
			Cell invasion	Notch1, Jagged1	Yes	Pang <i>et al.</i> (2010)
<i>miR-34a</i>	Cervical cancer tissue	↓				
<i>miR-375</i>	SiHa, CaSki cervical cancer cell lines		Cell proliferation, migration, invasion	SP1	Yes	Wang <i>et al.</i> (2011)
<i>miR-375</i>						
<i>miR-214</i>	Cervical cancer tissue	↓		ND	ND	Yang <i>et al.</i> (2009)
<i>miR-214</i>	HeLa cell line		Cell proliferation, migration, invasion	Plexin-B1	Yes	Qiang <i>et al.</i> (2011)

ND, not determined; CIN, cervical intraepithelial neoplasia; hEVEC, human ectocervical vaginal epithelial cells.

^aTarget validation was performed by 3'-UTR reporter construct assays and/or miR transfection and western analysis. ^b*miR-150* but not *miR-186a* targeted P2RX7 in HeLa cells.

miR-186 and *miR-150* inhibitors increased *P2RX7* mRNA levels in hEVEC cells, but only *miR-150* inhibitor increased *P2RX7* expression in HeLa cells. Collectively, these data suggest that the elevated levels of *miR-186* and *miR-150* in ectocervical cancerous tissue and cell lines may induce a downregulation of *P2RX7* and thereby an inhibition of apoptosis. Along these lines, *miR-886-5p* was also demonstrated to inhibit apoptosis and be over-expressed in cervical cancer tissue (Li *et al.* 2011a, 2011b). Forced expression of *miR-886-5p* in the cervical cancer cell line H8 was associated with reduced apoptosis and increased cell proliferation. This effect of *miR-886-5p* was demonstrated to be mediated via a direct effect on the pro-apoptotic protein, BAX. Collectively, these data demonstrate that over-expression of *miR-186*, *miR-150*, and *miR-886-5p* characteristic of cervical cancer functionally contributes to the pathogenesis by modulating the expression of antiapoptotic proteins.

In addition to cell proliferation, miRNAs have also been associated with cell invasion. Using an array of cervical and choriocarcinoma cell lines, Pang *et al.* (2010) demonstrated that *miR-34a* reduces cell invasion by targeting NOTCH1 and JAGGED1. Similarly, expression of *miR-375* was reported to be reduced in cervical cancer tissue (Wang *et al.* 2011). Forced expression of *miR-375* in both SiHa and CaSki cells was associated with reduced cell proliferation

(significant G1 arrest) and reduced migration and invasion. Associated with this *miR-375*-induced suppression of cell proliferation, migration, and invasion was a reduction in the expression of the transcription factor, SP1. Lastly, *miR-214* expression has also been reported to be reduced in cervical cancer and associated with enhanced cell growth (Yang *et al.* 2009). In a follow-up study by this group, Qiang *et al.* (2011) reported that *miR-214* expression was inversely correlated with that of plexin-B1 and a direct repressor of this target mRNA. Forced expression of *miR-214* in HeLa cells was also associated with reduced cell proliferation, migration, and invasion. Taken together, recent studies demonstrate that *miR-34a*, *miR-375*, and *miR-214* modulate cell invasion and their reduced expression in cervical cancer leads to enhanced cell migration, invasion, and proliferation.

In summary, the majority of our information on miRNA expression in the cervix is derived from studies using cancer specimens/cell lines. Of these, *miR-21* and *miR-143* appear to be the most well studied and both modulate cell proliferation. In cancer cells, *miR-21* is over-expressed while *miR-143* is under-expressed and their patterns of expression are reversed in normal cervical tissue/cells. In addition to our information on miRNAs gleaned from cancer studies, parturition studies have identified a subset of miRNAs (*miR-223*, *miR-34b*, and *miR-34c*), which are

associated with cervical ripening/parturition as well as cell proliferation and apoptosis. While the presented studies provide the initial miRNA expression profiles in this organ, future studies will need to emphasize dissecting the function of the miRNAs, their transcriptional targets, and the mechanisms by which these miRNAs regulate the cellular events within the cervix both from the normal function of this organ and from dysregulation of miRNAs and the development of cervical cancer.

Conclusions

Conditional deletion of the miRNA-processing enzyme DICER1 has revealed that miRNAs are essential for normal development and function of the mouse female reproductive tract. Additional studies have examined the expression, regulation, and function of miRNAs in the female reproductive tract of not only mice but also more of humans. miRNA expression profiles have been generated for ovary, oviduct, endometrium, myometrium, and cervix in both normal and diseased tissue as well as various cell lines. These studies not only provide a profile of miRNA expression but also to help understand what miRNAs may be misexpressed in diseases of the female reproductive tract and how dysregulation of these miRNAs contribute to the pathogenesis of the disease.

Overall, our understanding on the regulation of miRNA expression in the female reproductive tract is superficial. Studies are warranted to further define the roles of miRNAs in organ development, organ dysfunction, disease, and neoplasia. Additional emphasis should focus on identifying the precise mechanisms of miRNA expression in these processes. While we must appreciate the information obtained to date, we must also keep in mind that the majority of putative targets for those misexpressed miRNAs have yet to be validated. We must also exercise caution when extrapolating data from cancer cell lines to that of normal reproductive organ cells and tissues. To deepen our understanding, future approaches should focus on miRNA target identification, validation, and function of these miRNA transcript target pathways within each component of the female reproductive tract. This should start with localization of miRNAs of interest and concurrent assessment of those putative targets of relevancy in the same cells within each tissue. Patterns of expression for miRNA and protein should be inverse if in fact the specific miRNA is posttranscriptionally regulating the putative target. Next, it is essential to validate these mRNA targets, and this can be done through a combination of miRNA restoration/knockdown, western blot analysis, and 3'-UTR reporter constructs. As technology advances so will our ability to further dissect the mechanisms of miRNA regulation as well as the pathways that

these miRNAs regulate within the organs of the female reproductive tract.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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