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Cell-free nucleic acids as non-invasive biomarkers of gynecological cancers, ovarian, endometrial and obstetric disorders and fetal aneuploidy

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BACKGROUND: Proper folliculogenesis is fundamental to obtain a competent oocyte that, once fertilized, can support the acquisition of embryo developmental competence and pregnancy. MicroRNAs (miRNAs) are crucial regulators of folliculogenesis, which are expressed in the cumulus–oocyte complex and in granulosa cells and some can also be found in the bloodstream. These circulating miRNAs are intensively studied and used as diagnostic/prognostic markers of many diseases, including gynecological and pregnancy disorders. In addition, serum contains small amounts of cell-free DNA (cfDNA), presumably resulting from the release of genetic material from apoptotic/necrotic cells. The quantification of nucleic acids in serum samples could be used as a diagnostic tool for female infertility.

METHODS: An overview of the published literature on miRNAs, and particularly on the use of circulating miRNAs and cfDNA as non-invasive biomarkers of gynecological diseases, was performed (up to January 2014).

RESULTS: In the past decade, cell-free nucleic acids have been studied for potential use as biomarkers in many diseases, particularly in gynecological cancers, ovarian and endometrial disorders, as well as in pregnancy-related pathologies and fetal aneuploidy. The data strongly suggest that the concentration of cell-free nucleic acids in serum from IVF patients or in embryo culture medium could be related to the ovarian hormone status and embryo quality, respectively, and be used as a non-invasive biomarker of IVF outcome.

CONCLUSIONS: The profiling of circulating nucleic acids, such as miRNAs and cfDNA, opens new perspectives for the diagnosis/prognosis of ovarian disorders and for the prediction of IVF outcomes, namely (embryo quality and pregnancy).

Key words: MicroRNAs / cell-free DNA / infertility / non-invasive biomarkers

Introduction

MicroRNAs (miRNAs) are small (19–25 nucleotides), single-stranded, non-coding RNA molecules that bind specifically to, and post-transcriptionally regulate, several messenger RNAs (mRNAs) (Thomas et al., 2010). miRNAs play important physiological roles and miRNA dysregulation can lead to pathologies. In fertility, miRNAs are associated with the functional regulation of gonadal somatic cells [Leydig and Sertoli cells in testis, and granulosa and cumulus cells (CCs) in the ovary] involved in steroid synthesis. For example, in male mice, deletion of Dicer (a protein essential for miRNA maturation) in Sertoli cells leads to infertility due to the complete absence of spermatozoa and progressive testicular degeneration (Hossain et al., 2012). In female mice, Dicer inactivation leads to infertility due to multiple defects in ovarian functions, including abnormal cycles and an abnormal response to gonadotrophin (Follicle-stimulating hormone), leading to ovulation problem (Nagaraja et al., 2008).

During follicular development, oocytes are in close contact with the surrounding CCs to form the cumulus–oocyte complex (COC). The crosstalk between oocytes and CCs occurs through gap junctions (Albertini et al., 2001). This paracrine signaling is crucial for the acquisition of developmental competence in oocytes and CCs (Gilchrist et al., 2008). These reciprocal regulations are carefully modulated by some key genes that are themselves regulated by miRNAs (Assou et al., 2013a). Some miRNAs are found in body fluids and as they are contained in exosomes, they are highly stable because they are protected from RNases. The potential use of these circulating miRNAs as novel, non-invasive diagnostic/prognostic biomarkers is the focus of many investigations (Mitchell et al., 2008) and they are already used as biomarkers for the diagnosis and prognosis of several gynecological and pregnancy disorders (Carletti and Christenson, 2009).

Similarly, cell-free DNA (cfDNA) molecules, which are released mostly by apoptotic or necrotic cells, are also found in body fluids and can be used as biomarkers of pathological conditions (Schwarzenbach et al., 2011). Indeed, cfDNA has been detected in human semen (Chou et al., 2004). This cell-free seminal DNA contains DNA epigenetic information that is essential for proper spermatogenesis (Wu et al., 2013a). Circulating cfDNA in the bloodstream is also being used to detect gynecological abnormalities, whereas fetal cfDNA in maternal blood constitutes a non-invasive biomarker for fetal aneuploidy (Lo et al., 1999; Bischoff et al., 2002, 2005; Bauer et al., 2006; Lo and Chiu, 2008; Wright and Burton, 2009; Lambert-Messerlian et al., 2014).

In this review, we describe first the biogenesis of circulating cell-free miRNAs and DNA. Then, we present an analysis of the available data on circulating nucleic acids in gynecological diseases and in pregnancy and discuss their potential role in the 'oocyte–niche' crosstalk and in the hormonal regulation of folliculogenesis. Finally, we discuss the evidence suggesting that cell-free nucleic acids could be used as non-invasive biomarkers of IVF outcomes.

Methods

A summary of the general knowledge on cellular and circulating miRNAs was compiled based on seminal articles in this research field. A systematic review of the current literature in the English language on cellular and circulating nucleic acids (miRNAs and DNA) in relation to mammalian, including human, reproduction was performed. All the selected articles were searched in journal databases, such as PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez), using key words, including 'miRNA', 'circulating miRNA', 'cell-free DNA', 'oocyte', 'cumulus cells', 'embryo', 'pregnancy' and 'biomarkers'. The search retrieved *c.*10 000 articles; of which, 284 were included in this review.

Results

MicroRNAs

General considerations on cellular miRNAs

miRNAs belong to the 'small RNA' family and are evolutionarily conserved from invertebrates to vertebrates (Lagos-Quintana et al., 2001). miRNAs were first identified in *Caenorhabditis elegans* at the beginning of the 1990s (Lee et al., 1993). They are non-coding single-stranded RNA molecules of 19–25 nucleotides in length that arise from inter- or intragenic genomic regions. In mammals, miRNAs are usually complementary to a small region in the 3′ untranslated region (UTR) of mRNAs.

miRNAs are derived from primary transcripts (called pri-miRNAs) that are folded into hairpins and are synthesized via the classical transcription process using polymerase II (Lee et al., 2004; Rodriguez et al., 2004). Pri-miRNAs are then cleaved by a protein complex formed by Drosha (an enzyme of the RNase III complex) and its partner, the nuclear protein DiGeorge critical region 8 (DGCR8). This cleavage leads to ~70 nucleotide-long pre-miRNAs that are exported by Exportin 5 to the cytoplasm (Yi et al., 2003). Pre-miRNAs are then cleaved by Dicer, another protein of the RNase III complex, to eliminate the terminal loop and release double-stranded miRNAs of ~22 nucleotides (Hutvagner et al., 2001). Only one strand of each mature miRNA is then incorporated in the miRNA-induced silencing complex (miRISC), which includes either AGO1 or AGO2 proteins from the Argonaute family. In the miRISC complex, single-stranded miRNAs can interact with and silence their target mRNAs in two different ways. If the miRISC complex contains AGO2, the targeted mRNA is degraded. On the other hand, the presence of AGO1 in the RISC complex promotes translation repression (Hutvagner and Simard, 2008). Thus, miRNAs can regulate protein levels by promoting mRNA degradation and also by attenuating protein translation.

miRNAs are predicted to be involved in the silencing of more than half of mammalian genes (Friedman et al., 2009). Based on sequence homology, one single miRNA could regulate at least 200 mRNAs and consequently the expression of the corresponding proteins (Esquela-Kerscher and Slack, 2006). Some miRNAs are tissue-specific, while others are expressed in more than one tissue (Reedy et al., 2009). It is now acknowledged that miRNAs play a crucial role in the physiological regulation of many cellular processes. Moreover, miRNA expression must be very tightly and dynamically regulated to allow the specific modulation of different mRNAs, for instance during embryo development, cell transitions or cell environmental changes. In 2002, it was reported, for the first time, that deletion and down-regulation of specific miRNAs could be implicated in cancer (Calin et al., 2002). Further studies have demonstrated that miRNA mutations, biogenesis defects or deregulation can affect miRNA-mediated gene silencing, and this may result in serious diseases, such as cancers (Calin and Croce, 2006, Esquela-Kerscher and Slack, 2006), cardiovascular diseases (Latronico et al., 2007; van Rooij and Olson, 2007), neurological disorders (Esau and Monia, 2007; Fiore and

Schratt, 2007; Hansen *et al.*, 2007; Perkins *et al.*, 2007), ischemia (Silvestri *et al.*, 2009), heart failure (Adachi *et al.*, 2010; Ai *et al.*, 2010; Tijsen *et al.*, 2010; Wang *et al.*, 2010b), hepatitis (Wang *et al.*, 2012c), Crohn's disease, sepsis (Wang *et al.*, 2010c), tuberculosis (Singh *et al.*, 2013), diabetes (Farr *et al.*, 2013; Roberts and Porter, 2013) and obesity (Weiler *et al.*, 2006).

Circulating miRNAs in gynecological disorders and pregnancy

Most miRNAs are localized inside the cell; however, a significant number of miRNAs have been detected also in extracellular body fluids, such as serum, plasma, urine, spinal fluid, saliva and follicular fluid (Wang *et al.*, 2010b; Weber *et al.*, 2010; Zubakov *et al.*, 2010; Zen and Zhang, 2012; Sang *et al.*, 2013). These circulating miRNAs could be used as biomarkers of specific conditions, because they are relatively abundant (especially in blood) and quite stable due to their confinement within vesicles where they are protected from RNases. Both serum and plasma are suitable for the analysis of miRNAs (Mitchell *et al.*, 2008). miRNAs are selectively and actively secreted from cells and packaged into appropriate carriers. They are then transported to targeted or receptor-specific recipient cells where they recognize and repress mRNA targets within recipient cells (Boon and Vickers, 2013). miRNA intercellular transport is performed by different subclasses of miRNAs carriers, such as membrane-derived vesicles (exosomes and microparticules), lipoproteins and ribonucleoprotein complexes (Valadi *et al.*, 2007; Zhang *et al.*, 2010; Vickers *et al.*, 2011). Exosomes (small vesicles of 40–100 nm in diameter) and microparticles (100–4000 nm in diameter) have different biogenesis and secretory mechanisms (Thery, 2011). During apoptosis, cells can release even larger microparticles or apoptotic bodies to transport specific miRNAs (Zernecke *et al.*, 2009). Extracellular miRNAs can be transported also by high-density lipoproteins (HDLs) and low-density lipoproteins (LDLs), abundant in plasma (Vickers *et al.*, 2011). Biophysical studies have also shown that miRNAs can associate also with protein complexes, including AGO2, the main functional component of the cytoplasmic miRNA ribonucleoprotein complex (Arroyo *et al.*, 2011; Turchinovich *et al.*, 2011). Many observations suggest that miRNA export mechanisms are selective and regulated (Wang *et al.*, 2010d). For example, the miRNA profiles of extracellular vesicles are not representative of their parent cell type, but of specific sets of miRNAs. Indeed, the exosomal-, HDL- and LDL-miRNA signatures are distinct, although some miRNAs can be found in all carrier subclasses (Vickers *et al.*, 2011). Furthermore, specific miRNA profiles are consistent among individuals and each biological fluid has its own physiological miRNA signature (Valadi *et al.*, 2007; Vickers *et al.*, 2011). This suggests that a specific miRNA profile in serum or plasma could be associated with some pathological conditions. Since Lawrie *et al.* (2008) showed that the serum level of specific miRNAs was higher in patients with lymphoma than in healthy controls, cell-free miRNAs have been assessed in many different pathological conditions, including gynecological and pregnancy disorders, in order to identify tissue-specific miRNAs that may constitute non-invasive diagnostic tools. Moreover, as the amount of specific circulating miRNAs has been associated with tumor development and malignant progression (Schwarzenbach *et al.*, 2011), circulating cell-free nucleic acids are now used not only as diagnostic biomarkers, but also as prognostic tools.

Table I lists the circulating miRNAs used as biomarkers in gynecological disorders. For example, miR-205 expression is significantly upregulated and let-7f significantly lower in plasma samples from patients with epithelial ovarian cancer (EOC), especially in patients with Stage I EOC, compared with healthy controls (Zheng *et al.*, 2013). Likewise, miR-483-5p plasma level is higher in patients with Stage III and IV EOC than in those with Stage I and II EOC, consistent with its expression pattern in tumor tissues (Zheng *et al.*, 2013). miR-200a, miR-200b, miR-200c and miR-103 are significantly overexpressed in serum samples from patients with serous EOC compared with controls (Kan *et al.*, 2012). Moreover, circulating miR-92 is overexpressed in serum samples from patients with EOC compared with healthy controls (Guo *et al.*, 2013). Altogether, these findings suggest that, in the case of EOC, the concomitant evaluation of different circulating miRNAs might be used not only for early tumor detection, but also for its staging and prognosis.

In breast tumors, many miRNAs are differentially expressed in patients versus healthy women (Table I; Wang *et al.*, 2010a; Wu *et al.*, 2011; van Schooneveld *et al.*, 2012; Cuk *et al.*, 2013a,b). However, only seven are concomitantly overexpressed in the tumor and in serum. Among them, miR-1, miR-92a, miR-133a and miR-133b have been validated as the most important diagnostic markers for breast cancer (Chan *et al.*, 2013). Another study showed that also the serum level of miR-182 is significantly higher in patients with breast cancer compared with controls (Wang *et al.*, 2013a). In addition, miR-182 serum levels were considerably lower in patients with estrogen receptor- or progesterone receptor-positive breast tumors than in those with estrogen receptor- or progesterone receptor-negative cancers (Wang *et al.*, 2013a). All these data suggest that circulating miRNAs might also be used as biomarkers to diagnose and identify breast cancer type.

Recently, abnormal miRNA expression in the bloodstream has been associated also with several metabolic disorders, including obesity, diabetes and gynecological pathologies, such as polycystic ovary syndrome (PCOS), premature ovarian failure (POF) and endometriosis (Fernandez-Valverde *et al.*, 2011; Hulsmans *et al.*, 2011; Gilabert-Estelles *et al.*, 2012; Rottiers and Naar, 2012; Chen *et al.*, 2013). Indeed, miRNAs play a crucial role in metabolism regulation (Rottiers and Naar, 2012). For example, miR-33a and miR-33b, which are located within the sterol regulatory element-binding protein 1 and 2 (SREBP 1 and 2) genes, regulate cholesterol and lipid metabolism in concert with their host genes (Gerin *et al.*, 2010; Horie *et al.*, 2010; Marquart *et al.*, 2010; Najafi-Shoushtari *et al.*, 2010; Davalos *et al.*, 2011). Moreover, miR-103 and miR-107 are involved in controlling insulin and glucose homeostasis, and miR-34a is a key regulator of hepatic lipid homeostasis (Wilfred *et al.*, 2007; Takanabe *et al.*, 2008; Xie *et al.*, 2009; Trajkovski *et al.*, 2011). Therefore, circulating miRNAs may act as endocrine signaling molecules and could be used as potential biomarkers of metabolic diseases. For instance, it has been shown recently that miR-138, miR-15b and miR-376a might constitute reliable predictive biomarkers in obesity (Pescador *et al.*, 2013). Specifically, miR-138 and miR-376a could be used as a powerful predictive tool to differentiate obese patients from diabetic patients, obese diabetic patients and healthy controls. Moreover, miR-138 and miR-503 can differentiate between diabetic and obese diabetic patients (Pescador *et al.*, 2013).

PCOS is one of the most common endocrine disorders among women of reproductive age and is considered one of the leading causes of female infertility (Azziz *et al.*, 2004). The main features of PCOS are dysovulation (resulting in irregular menstrual cycles or amenorrhea and thus ovulation-related infertility), excessive levels of androgenic hormones (resulting in hirsutism) and insulin resistance, often associated with obesity, Type 2 diabetes and high cholesterol levels.

Table I Cell-free miRNA in human gynecological pathologies.

Gynecological pathology	Increased		Decreased	
	miRNAs	References	miRNAs	References
Ovarian cancer	miR-205	Zheng et al. (2013)	let-7f	Zheng et al. (2013)
	miR-483-5p (*Stages III and IV*)	Zheng et al. (2013)	miR-145	Wu et al. (2013b)
	miR-92	Guo et al. (2013)		
	miR-221	Hong et al. (2013)		
	miR-200a/b/c and miR-103	Kan et al. (2012)		
Breast cancer	miR-1, miR-92a, miR-133a and miR-133b	Chan et al. (2013)	miR-205	Liu et al. (2013)
	miR-182	Wang et al. (2010a)	miR-145	Ng et al. (2013)
	miR-148b, miR-376c, miR-409-3p and miR-801	Cuk et al. (2013a)	miR-92a	Si et al. (2013)
	miR-21 and miR-146a	Kumar et al. (2013)	miR-30a	Zeng et al. (2013)
	miR-34a, miR-93 and miR-373	Eichelser et al. (2013)	46 miRs	Schrauder et al. (2012)
	miR-155	Liu et al. (2013)	miR-181a	Guo and Zhang (2012)
	miR-10b, miR-21, miR-125b, miR-145, miR-155, miR-191 and miR-382	Mar-Aguilar et al. (2013)		
	miR-16, miR-21, miR-451	Ng et al. (2013)		
	miR-155	Sun et al. (2013)		
	miR-21	Si et al. (2013) and Asaga et al. (2011)		
	miR-20a and miR-21	Schwarzenbach et al. (2012)		
	13 miRs including miR-202	Schrauder et al. (2012)		
	miR-195	Heneghan et al. (2010)		
	miR-103	Wang et al. (2012a)		
Endometriosis	miR-199a and miR-122	Wang et al. (2013b)	miR-17-5p, miR-20a and miR-22	Jia et al. (2013)
			miR-141, miR-145 and miR-542-3p	Wang et al. (2013b)
PCOS	miR-21, miR-27b, miR-103 and miR-155	Murri et al. (2013)	miR-132 and miR-320[a]	Sang et al. (2013)
POF	miR-146a, miR-23a, miR-27a and miR-126	Yang et al. (2012)	let-7c and miR-144	Yang et al. (2012)

PCOS, polycystic ovary syndrome; POF, premature ovarian failure.
[a]In follicular fluid.

A recent study showed that miR-21, miR-27b, miR-103 and miR-155 levels are decreased in the bloodstream of obese men and women, whereas they are increased in women with PCOS compared with healthy controls (Murri *et al.*, 2013). These data suggest that the concomitant evaluation of different miRNAs might be used as biomarker to differentiate real obesity from obesity associated with PCOS.

Some miRNAs are differentially expressed in the plasma of women with POF compared with normal responder women (Table I). Some of these miRNAs may regulate granulosa cell proliferation and apoptosis by affecting different signaling pathways. For example, miR-23a may regulate apoptosis by decreasing *XIAP* expression in human granulosa cells (Yang *et al.*, 2012).

Moreover, miR-30b and miR-30d are significantly up-regulated in receptive endometrium, whereas miR-494 and miR-923 are down-regulated (Altmae *et al.*, 2013). In some endometrial disorders, endometrium receptivity can be altered and several studies have focused on the identification of miRNAs that may be deregulated in these diseases. Twenty-seven miRNAs were shown to be differentially expressed in women suffering from endometriosis in comparison with healthy controls (Jia *et al.*, 2013). miR-17-5p, miR-20a and miR-22 in particular were dramatically decreased in the plasma from patients with endometriosis compared with controls (Jia *et al.*, 2013). Moreover, the serum levels of miR-199a and miR-122 were higher in patients with endometriosis compared with controls, whereas miR-145, miR-141, miR-542-3p and miR-9 were lower (Wang *et al.*, 2013b). Finally, the relative expression of miR-199a and miR-122 has been used to discriminate between severe and mild endometriosis and thus constitutes a reliable biomarker to follow endometriosis progression (Wang *et al.*, 2013b).

The discovery of fetal miRNAs in the maternal bloodstream has paved the way to their possible use for non-invasive prenatal diagnosis. Specifically, placental miR-141, miR-149, miR-299-5p and miR-135b can be easily detected in maternal plasma during pregnancy and after delivery their plasma concentration significantly decreases (Chim *et al.*, 2008). In particular, miR-141 plasma level increases as pregnancy progresses into the third trimester. This promising new fetal biomarker appears to be more reliable for pregnancy monitoring than the currently used chorionic somatomammotropin hormone 1 mRNA level, because it is more stable in maternal plasma (Chim *et al.*, 2008).

Pre-eclampsia is one of the leading causes of maternal and fetal/neonatal mortality (Sibai *et al.*, 2005). One study showed that miR-210 level is up-regulated, whereas miR-152 is down-regulated in serum samples from patients with pre-eclampsia (Gunel *et al.*, 2011). Thus, miR-210 quantification in maternal serum could be used to improve pre-eclampsia diagnosis using non-invasive methods. Another study showed that miR-24, miR-26a, miR-103, miR-130b, miR-181a, miR-342-3p and miR-574-5p are significantly increased in plasma from pregnant women with severe pre-eclampsia (Wu *et al.*, 2012). The study of their target genes suggests that these miRNAs could be involved in many different functions, such as the regulation of metabolic processes, control of cell cycle and signaling pathways, including the mitogen-activated protein

kinase and the transforming growth factor-β (TGF-β signaling pathways), or pathways involved in cancer metastasis. In addition, they could also play important roles in pre-eclampsia development and its severity and might constitute potential biomarkers for this disease (Wu *et al.*, 2012). Moreover, some miRNAs, particularly miR-323-3p, could improve the accuracy of ectopic pregnancy detection in association with plasma hCG and progesterone levels (Zhao *et al.*, 2012).

In conclusion, due to their accessibility and stability (miRNAs circulate confined within exosomes), different circulating miRNAs could be used, alone or in combination, as non-invasive biomarkers of gynecological cancers and gynecological disorders.

Role of miRNAs in the oocyte–niche relationship and in the hormonal regulation of folliculogenesis

During the early stages of follicular development, a specific crosstalk between the oocyte and follicular cells is established. At the pre-antral secondary stage, follicular cells differentiate into two types: granulosa cells that cover the follicle and CCs that are directly in contact with the oocyte. Then, the COC is formed and the oocyte–CC dialog is organized via tight junctions. Oocyte secreting factors participate in CC differentiation and proliferation. Reciprocally, CCs provide nutrients for the oocyte development and maturation (Mori *et al.*, 2000; Eppig, 2005; Sugiura *et al.*, 2005; Gilchrist *et al.*, 2008). These mutual regulations are mainly controlled by the growth differentiation factor-9 (GDF9) and the bone morphogenetic protein 15 (BMP15) genes that belong to the TGF-β family and are expressed in the oocyte. *GDF9* induces the expression of many genes in CCs, including genes implicated in oocyte maturation and quality, such as Hyaluronic Acid Synthase 2 (*HAS2*), and genes involved in fertilization and embryo development, such as Cyclo-Oxygenase 2 (COX-2) and *Gremlin 1* reviewed in Gilchrist *et al.* (2008) and Assou *et al.* (2010). Therefore, oocyte quality and its ability to contribute to the formation of a 'competent' embryo with a strong potential to implant in the endometrium and lead to a successful pregnancy can be predicted by using indirect CC markers. miRNAs have been identified in human oocytes (Xu *et al.*, 2011b; Assou *et al.*, 2013a). In mouse, oocyte miRNAs are not essential for meiosis (Suh *et al.*, 2010). Indeed, Dgcr8 is required only for miRNA processing, whereas Dicer is also implicated in small interfering RNA (siRNA) processing. Dicer loss in mouse oocytes results in meiotic arrest and severe spindle and chromosomal segregation defects, whereas Dgcr8 loss showed no phenotype (Murchison *et al.*, 2007; Tang *et al.*, 2007; Suh *et al.*, 2010). This suggests that siRNAs rather than miRNAs are involved in oocyte meiosis. On the other hand, miRNAs also could have a major role in the regulation of follicular cell functions, such as steroidogenesis, apoptosis, luteinization as well as in ovulation process (Hawkins and Matzuk, 2010). For example, treatment of mouse mural granulosa cells with LH leads to the deregulation of a set of miRNAs (particularly miR-132 and miR-212 overexpression) that are possibly important for the control of ovarian functions (Fiedler *et al.*, 2008). Overexpression of miR-93 could disturb ovary development. Indeed, miR-93 targets the mRNA encoding LHX8, a protein that contains a Lim homeodomain required for the transition from primordial to primary follicle (Pangas *et al.*, 2006).

Table II lists the miRNAs found in the COC, granulosa cells, the follicular fluid and the corpus luteum. Table III summarizes the miRNAs involved in folliculogenesis and Table IV the miRNAs involved in hormonal regulations.

Many studies have shown that hormones from the hypothalamic–pituitary–gonadal axis, which are essential for sexual maturation and reproductive function in mammals, are also involved in the regulation of some miRNAs. Gonadotropin-releasing hormone (GnRH) stimulates the synthesis and the secretion of the pituitary gonadotrophins Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH) that then regulate the production of gonadal steroids and gametogenesis (Conn and Crowley, 1994; Kaiser *et al.*, 1997). GnRH also induces the expression of multiple miRNAs, particularly miR-132 and miR-212, which are encoded by the same gene that is induced by GnRH (AK006051) (Godoy *et al.*, 2011). LH acts on ovarian granulosa cells to induce ovulation and luteinization, resumption of oocyte meiosis and CC expansion that are crucial steps for ovulation. Moreover, LH acts as a survival factor by preventing apoptosis of granulosa cells (Robker and Richards, 1998; Chaffin *et al.*, 2001). Interestingly, LH also up-regulates miR-132, miR-212 and miR-21 in mural granulosa cells (Fiedler *et al.*, 2008). miR-21 is overexpressed in many tumors, including breast, pancreatic, colorectal and esophageal cancer, and thus is considered as an onco-miRNA (Cho, 2007; Dillhoff *et al.*, 2008; Verghese *et al.*, 2008). miR-21 depletion induces caspase-dependent apoptosis of mouse granulosa cells *in vitro* and *in vivo* (Carletti *et al.*, 2010), highlighting the physiological anti-apoptotic role of miR-21 in normal tissues. miR-200b and miR-429 depletion inhibits LH synthesis by repressing transcription of the gene encoding the β subunit of LH. This results in a lower serum LH concentration and the absence of the LH surge, leading to ovulation failure (Hasuwa *et al.*, 2013). Thus, the hypothalamic–pituitary–ovarian axis requires miR-200b and miR-429 to ensure ovulation. Finally, miR-122 is involved in the down-regulation of LH receptor expression by increasing the expression of LH receptor mRNA-binding protein via activation of SREBPs (Azhar, 2013; Menon *et al.*, 2013).

FSH has a crucial role in both follicle development and granulosa cell proliferation and differentiation. Several miRNAs, including miR-143, miR-125b, miR-21 and the let-7 family, are involved in follicular development in the mouse (Yao *et al.*, 2009). The expression of these RNAs is very low in primordial follicles, but they become readily detectable in granulosa cells of primary, secondary and antral follicles. miR-143, let-7a and miR-15b are negatively regulated by FSH (Yao *et al.*, 2009). Moreover, miR-133b is involved in FSH-induced estrogen production, by binding to the 3′ UTR of *Foxl2* and thus reducing FOXL2 protein level in granulosa cells (Dai *et al.*, 2013). FOXL2 is expressed in the ovaries and is necessary for granulosa cell function (Schmidt *et al.*, 2004), particularly through regulation of steroidogenesis genes, including StAR and CYP19A1 that are essential for promoting estradiol production (Pisarska *et al.*, 2011; Caburet *et al.*, 2012).

The involvement of miRNAs in the hormonal regulation during folliculogenesis and in the oocyte–niche crosstalk could be exploited for identifying new non-invasive biomarkers of fertility. Moreover, the development of therapies that block the expression or mimic the functions of specific miRNAs may represent a new therapeutic strategy for many gynecological disorders.

Circulating cell-free DNA

Biology of circulating cfDNA

DNA fragments are found in the blood circulation. Circulating cfDNA are double-stranded molecules with low molecular weight than genomic DNA, in the form of short fragments (between 70 and 200

Table II miRNAs expressed in the COC, GCs, FF or CL.

miRNAs	Expression	Species	Regulation	Target genes	Functions	References
COC						
miR-205, miR-150, miR-122, miR-96, miR-146a and miR-146b-5p	Oocyte	Bovine	–	–	Oocyte maturation Dynamic degradation during oocyte bovine maturation	Abd El Naby *et al.* (2013)
let-7b and let-7i miR-106a	COC	Bovine	–	MYC WEE1A	Oogenesis	Miles *et al.* (2012)
let-7b, let-7c, miR-27a and miR-322	CC (IVM)	Mouse	–	IGFBP-2	Oocyte meiotic competence	Kim *et al.* (2013)
miR-335-5p	Oocyte	Mouse	–	Actin nucleator Daam1 ERK1/2 Mitogen-activated protein kinase pathway	Oocyte meiosis Cytoskeleton dynamics Spindle formation	Cui *et al.* (2013)
Dicer1 miR-103, miR-16, miR-30b, miR-30c and let-7d	Oocyte (*Dicer depletion*)	Mouse	–	–	Oogenesis Meiosis: meiotic spindle organization and chromosome congression Oocyte meiotic maturation	Murchison *et al.* (2007) Tang *et al.* (2007) Choi *et al.* (2007b)
32 miRs including miR-23	CCs	Human	–	BCL2 CYP19A1	Apoptosis Steroidogenesis	Assou *et al.* (2013a)
miR-184 miR-10A miR-100	Oocyte	Human	–	NCOR2 HOXA1 SMARCA5	Transcriptional repression activity of nuclear receptors Regulation of oocyte-specific gene expression Oocyte reprogramming	Assou *et al.* (2013a)
miR-15a and miR-20a	Oocyte	Human	FSH?	BCL-2 family members and CDC25A	Oocyte maturation	Xu *et al.* (2011b)
GC						
30 miRs including miR-409a and miR-355 35 miRs including miR-183 cluster	GC in dominant follicles GC in subordinate follicles	Bovine	– –	*Targets of miR-409a:* BCL2L11, BIRC5, PTEN, Wnt, MAPK, TGF-β signaling	Apoptosis, cell proliferation, migration and differentiation Follicular development and atresia	Gebremedhn *et al.* (2013)
miR-26b	GC	Pig	–	ATM	Pro-apoptotic role Follicular atresia	Lin *et al.* (2012)
miR-23b, miR-29a and miR-30d	GC	Rat	Regulation by FSH	*Targets of miR-29a:* COL4A1 and BMF *Targets of miR-30d:* RNF2 and EED	–	Yao *et al.* (2010a)
miR-181a	GC	Mouse	–	ACVRIIA	Supression of GC proliferation	Zhang *et al.* (2013c)
miR-145	GC	Mouse	–	ACVRIB, CCND2	Supression of GC proliferation	Yan *et al.* (2012)
miR-224	Pre-antral GC	Mouse	Up-regulation by TGF-β1/SMAD pathway	SMAD4	GC proliferation Ovarian estrogen release (CYP19A1)	Yao *et al.* (2010b)
miR-21	Mural GC	Mouse	Up-regulation by hCG	PDCD4, PTEN, tropomyosin-1 and sprouty homolog 2	Anti-apoptotic role	Carletti *et al.* (2010) Fiedler *et al.* (2008)

Continued

Table II *Continued*

miRNAs	Expression	Species	Regulation	Target genes	Functions	References
miR-503	GC	Mouse (*Amhr2-Dicer1*)	Stimulation by gonadotrophins	Down-regulation of ACVR2a (ActRIIa), ACVR2b (ActRIIb), FSHR, BCL2 and CCND2	Ovarian development Proliferation during folliculogenesis (down-regulation during early follicular development, increase during later stage before ovulation and decline during luteinization)	Lei *et al.* (2010) Nagaraja *et al.* (2008)
miR-132 and miR-212	Mural GC	Mouse	Up-regulation by hCG	77 mRNA, CTBP1 protein synthesis	Ovarian function of CTBP1 unknown	Fiedler *et al.* (2008)
miR-23a	GC	Human	–	XIAP Caspase-3	Pro-apoptotic role	Yang *et al.* (2012)
miR-21	GC lines (KGN)	Human	–	COL4A1 mRNA	Basement membrane surrounding the GC layer and granulosa-embedded extracellular structure	Mase *et al.* (2012)
Pre-miR-10a, miR-105 and miR-182 miR-15a	GC	Human	–	CyclinB1 TdT, caspase-3 PCNA	Apoptosis and cell proliferation	Sirotkin *et al.* (2010)
FF						
miR-654-5p miR-640 miR-526b miR-373	GC transfected with exosomes	Bovine	–	ITGA3 SOCS4 MAP3K1 BRMS1L ZNFX1 CD44 VEGFA	Tumor progression (melanoma) Apoptosis and cell proliferation (retinal development) Ovarian primordial follicle activation Neonatal development Early endometrial response to pregnancy Network of matrices in COC extracellular space Neovascularization and vascular permeability during pre-antral follicle development	Sohel *et al.* (2013)
miR-181A, miR-375 and miR-513a-3p	FF	Equine	–	TGF-β signaling (24 genes)	Follicle development and growth Oocyte maturation	Da Silveira *et al.* (2012)
miR-222, miR-193b and miR-520c3p miR-191, miR-483-5p, miR-146a, miR-320, miR-24, miR-574-3p, miR-1290 and miR-518a miR-132, miR-24 and miR-320 miR-24 miR-132, miR-320 and miR-520-3p miR-222, miR-24, miR-193b and miR-483-5p	FF FF	Human Human	– –	PTEN, ESR1 IL-1A, IL-10, IL-12B, IL-37, IL-8 TGF-β1 PDK3 HMGA2, RAB5B TGF-β1	Tumor suppressor, negative regulation of insulin signaling and glucose metabolism in adipose tissue Steroidogenesis process Immune system Reproductive aging, cell proliferation, metabolic diseases Regulation of glucose metabolism PCOS (Shi *et al.* (2012) Steroidogenesis	Sang *et al.* (2013)

Continued

Table II *Continued*

miRNAs	Expression	Species	Regulation	Target genes	Functions	References
CL						
miR-378	Non-regressed and regressed CL	Bovine	–	IFNGR1	Luteal cell apoptosis (increase during luteal development and decrease during luteal regression)	Ma *et al.* (2011)
miR-125b miR-145 miR-199a-3p miR-503	Theca cells and CL	Sheep		LIF CDKN1A PTGS2 –	Luteinization (decrease in luteinization during the follicular–luteal transition) Decrease in per-ovulatory follicles and increase in CL	McBride *et al.* (2012)
Let-7b and miR-17-5p	CL	Mouse (*mutant with Dicer1 hypomorphic allele*)	–	TIMP 1	CL formation and function Maintain pregnancy Angiogenesis	Otsuka *et al.* (2008)

IVM, *in vitro* maturation; COC, cumulus–oocyte complex; GC, granulosa cell; FF, follicular fluid; CL, corpus luteum.

base pairs in length) or long fragments up to 21 kb. Two different mechanisms (not mutually exclusive) could explain the presence of cfDNA in the blood circulation. The first one is a passive mechanism due to the release of nuclear and mitochondrial DNA during the destruction of apoptotic and necrotic cells (Schwarzenbach *et al.*, 2011). In normal conditions, cell debris is phagocytozed by macrophages and thus the cfDNA level in blood remains low in healthy individuals (Pisetsky and Fairhurst, 2007). However, after phagocytosis of necrotic cells, DNA might be partially released into the bloodstream inside nucleosomes where it is protected from enzymatic degradation (Holdenrieder *et al.*, 2001a,b). This mechanism occurs in both healthy individuals and patients with benign diseases. The second mechanism is an active one probably through cell secretion (Gahan *et al.*, 2008). Many studies have reported high concentrations of cfDNA in plasma or serum of patients with cancer or other severe diseases (Laktionov *et al.*, 2004). Moreover, recent studies using genome-wide sequencing of plasma DNA have revealed that circulating tumor DNA represents the tumor genome and reflects the clonal genomic evolution of cancers (Murtaza *et al.*, 2013). Circulating cfDNA should be rapidly degraded by nucleases, and it has been shown that mutated cfDNA is degraded more rapidly than non-mutated cfDNA (Diehl *et al.*, 2005).

Circulating cfDNA for the non-invasive diagnosis of gynecological and pregnancy disorders

Changes in the levels of circulating DNA have been associated with several diseases, including gynecological and fetal disorders (Table V).

cfDNA could be used for the early detection and monitoring of gynecological malignancies. For example, circulating cfDNA can be measured to detect EOC at early stages (Zhang *et al.*, 2013b). The total cfDNA concentration in blood samples from patients with ovarian cancer is higher, particularly at advanced stages of the disease, than in healthy controls (Kamat *et al.*, 2006b). Very high pre-operative plasma levels of cfDNA are significantly associated with decreased patients' survival and constitute an independent predictor of death from ovarian cancer (Kamat *et al.*, 2010; No *et al.*, 2012). EOC is rarely detected early and it is not easy to determine whether an adnexal mass is malignant or benign. Interestingly, patients with EOC or endometriosis have significantly different levels of circulating cell-free mitochondrial DNA, but not of circulating cell-free nuclear DNA (Zachariah *et al.*, 2008). cfDNA originating from promotors can be methylated. The methylation profile of this cfDNA could also be used to differentiate between some benign and malignant tumors (Liggett *et al.*, 2011a,b). The level of tumor-specific DNA in plasma increases progressively with the tumor burden. On the other hand, it can decrease following chemotherapy. Indeed, tumor-specific plasma DNA levels were significantly higher in mice without treatment compared with animals treated with a combination of cytotoxic chemotherapy and anti-angiogenic agents against ovarian carcinoma (Kamat *et al.*, 2006a). Thus, tumor-specific cfDNA may be a useful biomarker of therapeutic response as well. This was confirmed by a recent paper showing that exome-wide analysis of circulating tumor DNA could complement the current invasive biopsy approaches to identify mutations associated with acquired drug resistance in advanced cancer (Murtaza *et al.*, 2013).

In the case of endometrial cancer, measurement of cfDNA is not useful for the detection of this malignancy. However, changes in cfDNA levels in a given patient after surgery/drug treatment may be a prognostic biomarker (Tanaka *et al.*, 2012).

DNA isolated from maternal blood is a mixture of fetal and maternal DNA in proportions that change during pregnancy progression. Although it is called fetal DNA, it derives from apoptotic placental cells (Huppertz and Kingdom, 2004; Hahn *et al.*, 2005).

Maternal obesity is associated with increased circulating total cfDNA, but not with fetal cfDNA. This could be due to less efficient clearance of cfDNA in obese women (Vora *et al.*, 2012). However, it is more likely to be the result of increased production of total cfDNA because decreased clearance would also lead to an increase in fetal cfDNA. In obese pregnant women, active remodeling of adipose tissue via adipocyte necrosis and/or apoptosis of the stromal vascular fraction results in higher release of cfDNA of maternal origin in the circulation (Haghiac *et al.*, 2012). In addition, the total cfDNA level is correlated with the maternal BMI and the gestational weigh gain (Lapaire *et al.*, 2009; Haghiac *et al.*, 2012).

Circulating cfDNA levels are higher in pregnant women with pre-eclampsia or abnormal placental invasion, as reported by different studies using real-time quantitative PCR for the male-specific SRY (sex-

Table III miRNAs implicated in folliculogenesis.

miRNAs	Expression	Species	Regulation	Target genes	Functions	References
miR-143	Ovary	Mouse	–	Genes related to the cell cycle	Primordial follicle formation Suppression of pre-granulosa cell proliferation	Zhang *et al.* (2013a)
miR-145	Neonatal ovary	Mouse	–	*Tgfbr2* SMAD signaling	Initiation of primordial follicle development and maintenance of primordial follicle quiescence	Yang *et al.* (2013)
Dicer-1	Oviductal, uterine mesenchyme, granulosa cells from pre-antral and small antral follicles	Mouse (*Amhr2-Dicer1*)	–	Follicle development-related genes such as *Amh*, *Inhba*, *Cyp17a1*, *Cyp 19a1*, *Zps*, *Gdf9*, *Bmp15*	Reproductive tract abnormalities (primary oviductal defect leading to infertility) Follicle cell proliferation, differentiation and apoptosis Follicle development and atresia (accelerated early follicle recruitment and reduction in the number of pre-ovulatory follicles) Oocyte maturation Estrous cycle: reduction in the number of natural or induced ovulations	Lei *et al.* (2010) Gonzalez and Behringer (2009) Pastorelli *et al.* (2009) Hong *et al.* (2008) Nagaraja *et al.* (2008)
miR-125b miR-21 let-7 family let-7a miR-143 miR-15b	Granulosa cells	Mouse	– Negative control by FSH	–	*Follicular development:* low expression in primordial follicles and increased expression in primary, secondary and antral follicles	Yao *et al.* (2009)
miR-709	Ovary	Newborn mouse	–	*Nobox*	Folliculogenesis (transition from primordial to primary follicle stage) Oogenesis	Choi *et al.* (2007b)
miR-93	Ovary	Mammalian	–	*Lhx8*	Folliculogenesis (transition from primordial to primary follicle stage) Oogenesis	Zhao and Rajkovic (2008) Pangas *et al.* (2006)

determining region of Y chromosome) or DYS 14 loci (Y chromosome-specific DNA sequence 14) (Zhong *et al.*, 2001; Sekizawa *et al.*, 2004). Levine *et al.* (1997, 2004), using blood samples from 120 women who developed pre-eclampsia and from 120 controls with normal pregnancy, showed a significant increase of fetal cfDNA levels, starting from Week 17 of gestation, in women who subsequently developed eclampsia compared with gestational age-matched controls. Several other studies confirmed that circulating fetal cfDNA levels are significantly elevated in pregnancies complicated by pre-eclampsia (Leung *et al.*, 2001; Zhong *et al.*, 2002; Azziz *et al.*, 2004; Levine *et al.*, 2004; Lazar *et al.*, 2009, 2010).

Fetal cfDNA is detectable in the plasma of pregnant women up to few hours after birth and could thus be used for non-invasive prenatal testing to detect chromosomal abnormalities (Hui and Bianchi, 2013). Indeed, fetal cfDNA is considered a reliable non-invasive biomarker of fetal aneuploidy (Bischoff *et al.*, 2002, 2005; Azziz *et al*, 2004; Bauer *et al.*, 2006; Wright and Burton, 2009; Abd El Naby *et al.*, 2013; Canick *et al.*, 2013). First, the presence of fetal cfDNA in the amniotic fluid was explored as a non-invasive method for the early detection of fetal chromosomal abnormalities. Then, it was demonstrated that specific fetal aneuploidies, such as trisomy 13, 18 or 21, can be detected in fetal cfDNA from maternal serum samples (Dan *et al.*, 2012; Norton *et al.*, 2012; Palomaki *et al.*, 2012; Sparks *et al.*, 2012; Zimmermann *et al.*, 2012; Fairbrother *et al.*, 2013; Nicolaides *et al.*, 2014). Moreover, fetal cfDNA in maternal plasma is also used to detect pathogenic copy number variations using target region capture sequencing, for instance in the case of family history of thalassemia (Ge *et al.*, 2013). Fetal cfDNA is used also for fetal sex determination in pregnant women who are carriers of X-linked genetic disorders in order to avoid invasive chorionic villus sampling, generally performed at 11–13 week of gestation (Miura *et al.*, 2011; Abd El Naby *et al.*, 2013).

Fetal cfDNA enrichment in amniotic fluid and in the maternal bloodstream, detected by using the improved new technologies, will contribute

Table IV miRNAs implicated in hormonal regulation.

miRNAs	Expression	Species	Regulation	Target genes	Functions	References
miR-378	GC (*in antral follicle growth*)	Pig		Aromatase (*identification of two binding sites in the 3′ UTR of the aromatase coding sequence*)	Inhibition of CYP19A1 (post-transcriptional down-regulation of aromatase expression) Estradiol production *in vitro*	Xu *et al.* (2011a)
miR-122	Ovary	Rat	Up-regulation by hCG (*activation of CAMP/PKA/ERK*)	LHR mRNA-binding protein	–	Menon *et al.* (2013) Azhar (2013)
miR-133b	GC	Mouse	–	*Foxl2*	Inhibition of Foxl2-mediated transcriptional repression of StAR and CYP19A1 Stimulation of estradiol production	Dai *et al.* (2013)
miR-200b and miR-429	Pituitary gland	Mouse	–	*Zeb1*	Support ovulation	Hasuwa *et al.* (2013)
miR-383	GC (*culture in vitro*) and oocyte	Mouse	Down-regulation by TGF-β1 in pre-antral follicles Up-regulation by gonadotrophins in antral follicles Decrease before ovulation transcriptional activation by SF-1	*Rbms1* (DNA-binding protein that activates MYC)	Stimulation of CYP19A1 and estradiol levels	Yin *et al.* (2012) Parker and Schimmer (1997)
miR-224	Pre-antral GC	Mouse	Up-regulation by TGF-β1/SMAD pathway	*Smad4*	GC proliferation CYP19A1 stimulation Ovarian estrogen release	Yao *et al.* (2010a)
miR-24 miR-132, miR-320 and miR-520-3p miR-222, miR-24, miR-193b and miR-483-5p	FF	Human	–	*TGF-β1* – –	Decrease in estradiol secretion Stimulation of estradiol secretion Decrease in progesterone secretion	Sang *et al.* (2013)
51 miRs 36 or 57 miRs 10 miRs miR-15a and miR-188 miR-107	GC (culture *in vitro*)	Human	–	–	Suppression of estradiol production Inhibition of progesterone release Stimulation of progesterone release Induction of progesterone output Increase in progesterone output	Sirotkin *et al.* (2009)

to more sensitive and accurate prenatal diagnosis in the near future and might greatly extend the scope of non-invasive prenatal diagnosis.

Nucleic acids as emerging non-invasive diagnostic biomarkers of female infertility

Circulating miRNAs and ovarian function

Several studies have shown that miRNAs are involved in intercellular signaling (Valadi *et al.*, 2007). In order to identify the miRNAs that are implicated in the CC–oocyte crosstalk and that regulate key genes implicated in folliculogenesis and ovarian function, we analyzed by deep sequencing the miRNAs present in mature metaphase II oocytes and in the associated CCs (Assou *et al.*, 2013a). Only 3 miRNAs were found to be expressed in oocytes (miR-184, miR-100 and miR-10a) and 32 in CCs.

Among the miRNAs expressed in the COC (Assou *et al.*, 2013a), some of them were found also in the plasma (Rekker *et al.*, 2013) (Fig. 1). It could be interesting to select some of these miRNAs, based on their possible involvement in folliculogenesis, and investigate whether their blood level could be used as a marker of IVF outcome. Moreover, the possible

Table V cfDNA in gynecological pathologies and fetal cfDNA in the maternal circulation.

	Pathologies	References
cfDNA	Ovarian cancer	Zhang *et al.* (2013b); Kamat *et al.* (2010); Liggett *et al.* (2011a); Dobrzycka *et al.* (2011); Kamat *et al.* (2006a, b)
	Endometrial cancer	Tanaka *et al.* (2012); Dobrzycka *et al.* (2010)
	Maternal obesity	Vora *et al.* (2012); Haghiac *et al.* (2012); Lapaire *et al.* (2009)
	Pre-eclampsia/HELLP syndrome	Miranda *et al.* (2013); Lazar *et al.* (2010); Lazar *et al.* (2009); Swinkels *et al.* (2002)
Fetal cfDNA	Pre-eclampsia/HELLP syndrome	Hahn *et al.* (2011); Lazar *et al.* (2010); Lazar *et al.* (2009); Levine *et al.* (2004); Bianchi (2004); Cotter *et al.* (2004); Zhong *et al.* (2002); Hahn and Holzgreve (2002); Swinkels *et al.* (2002); Zhong *et al.* (2001); Leung *et al.* (2001); Lo *et al.* (1999)
	Abnormal placental invasion	Sekizawa *et al.* (2002)
	Pre-term delivery	Leung *et al.* (1998)
	Aneuploidy	Horsting *et al.* (2014); Nicolaides *et al.* (2014); Russo and Blakemore (2014); Robinson *et al.* (2014);
	Trisomy (13, 18, 21)	Bianchi and Wilkins-Haug (2014); Gorzelnik *et al.* (2013); Verweij *et al.* (2013); Benn *et al.* (2013); Walsh and Goldberg (2013); Canick *et al.* (2013); Langlois *et al.* (2013); Shea *et al.* (2013); Webb and Murphy (2012); Hou *et al.* (2012); Wang *et al.* (2012a); Dan *et al.* (2012); Lazar *et al.* (2012); Wright and Burton (2009); Lo and Chiu (2008); Zimmermann *et al.* (2008); Montagnana *et al.* (2007); Deng and Li (2007); Bischoff *et al.* (2005); Bianchi (2004); Wataganara and Bianchi (2004); Spencer *et al.* (2003); Farina *et al.* (2003); Wataganara *et al.* (2003); Lee *et al.* (2002); Bischoff *et al.* (2002); Ohashi *et al.* (2001); Zhong *et al.* (2000); Lo *et al.* (1999)
	Fetal sex determination (X-linked genetic disorders)	Khorram Khorshid *et al.* (2013); Wright *et al.* (2012); Hou *et al.* (2012); Hill *et al.* (2012); Miura *et al.* (2011); Zimmermann *et al.* (2008); Deng and Li (2007); Montagnana *et al.* (2007); Lo (2005); Chen *et al.* (2004); Honda *et al.* (2002); Costa *et al.* (2002); Sekizawa and Saito (2001); Costa *et al.* (2001)
	α-Thalassemia	Ge *et al.* (2013); Sirichotiyakul *et al.* (2012)
	β-Thalassemia	Li *et al.* (2011); Chen *et al.* (2008); Lo (2005); Chiu *et al.* (2002b)
	Achondroplasia	Lim *et al.* (2011); Saito *et al.* (2000)
	Myotonic dystrophy	Amicucci *et al.* (2000)
	Cystic fibrosis	Gonzalez-Gonzalez *et al.* (2005); Gonzalez-Gonzalez *et al.* (2002)
	Huntington's disease	Gonzalez-Gonzalez *et al.* (2003)
	Congenital adrenal hyperplasia	Rijnders *et al.* (2001); Chiu *et al.* (2002a)
	Hemolytic disease of fetus and newborn	Illanes and Soothill (2009)

cfNDA, cell-free DNA; HELLP: hemolysis, elevated liver enzymes, low platelets.

relationship between hormonal markers of ovarian reserve/function, at Day 3 of the cycle and circulating miRNA expression could also be assessed. Interestingly, miR-30d expression is found altered in rat granulosa cells in culture after incubation with FSH (Yao *et al.*, 2010b). Moreover, miR-320a was reported to be decreased in follicular fluid from patients with PCOS (Sang *et al.*, 2013). It could be important to determine whether this decrease is correlated with LH or anti-Müllerian hormone (AMH) levels, which are higher in patients with PCOS. AMH is expressed by granulosa cells and controls the development of antral follicles by inhibiting excessive follicular recruitment by FSH (Weenen *et al.*, 2004). AMH is a marker of ovarian reserve and of associated pathologies, such as PCOS and POF (Visser *et al.*, 2006) and is commonly used to predict the ovarian response before IVF procedures (Broer *et al.*, 2013). Furthermore, some patients' characteristics, such as BMI, could also be compared with circulating miRNA levels since they can have an influence on hormonal regulation. Indeed, high BMI is considered as an indicator of female infertility and the deregulation of some miRNAs has been implicated in obesity (Hulsmans and Holvoet, 2013).

More generally, circulating miRNAs might represent an as yet unexplored tool for the diagnosis/monitoring of infertility/ovarian response.

cfDNA as a biomarker of ovarian function

cfDNA is released into the circulation following physiological and pathological cell necrosis and apoptosis. Based on the finding that the abundance of cfDNA can change in abnormal situations, we hypothesized that variations, particularly an increase, in circulating cfDNA might reflect ovarian reserve disorders. cfDNA is easily quantifiable in serum, and in women undergoing IVF it would be interesting to compare cfDNA concentration with FSH, LH, AMH and estradiol levels at Day 3 of the cycle, patient characteristics and IVF outcome. Recently, it was reported that increased plasma cfDNA levels are associated with low pregnancy rates in IVF programmes (Czamanski-Cohen *et al.*, 2013). However, the only correlation was between cfDNA and pregnancy outcome, once the patient was pregnant. The same group also reported a reduction in the higher than normal plasma cfDNA levels in a group of women undergoing IVF procedures following the practice of stress-reduction techniques, suggesting that these techniques may facilitate physiological changes leading to a reduction in plasma cfDNA levels and ultimately an improved IVF outcome (Czamanski-Cohen *et al.*, 2014).

miRNA and cfDNA in embryo culture medium

miRNAs are involved in the regulation of mammalian embryo development (Foshay and Gallicano, 2009; Medeiros *et al.*, 2011). Global miRNA expression profiling suggests that miRNA synthesis and degradation dynamically coexist during preimplantation embryo development (Yang *et al.*, 2008). In addition, intracellular miRNAs might modulate the transition of human embryonic stem cells (hESCs) to the differentiated cells that form the early germ layers (Wong *et al.*, 2012). Many miRNAs are expressed in developing mammalian embryos and

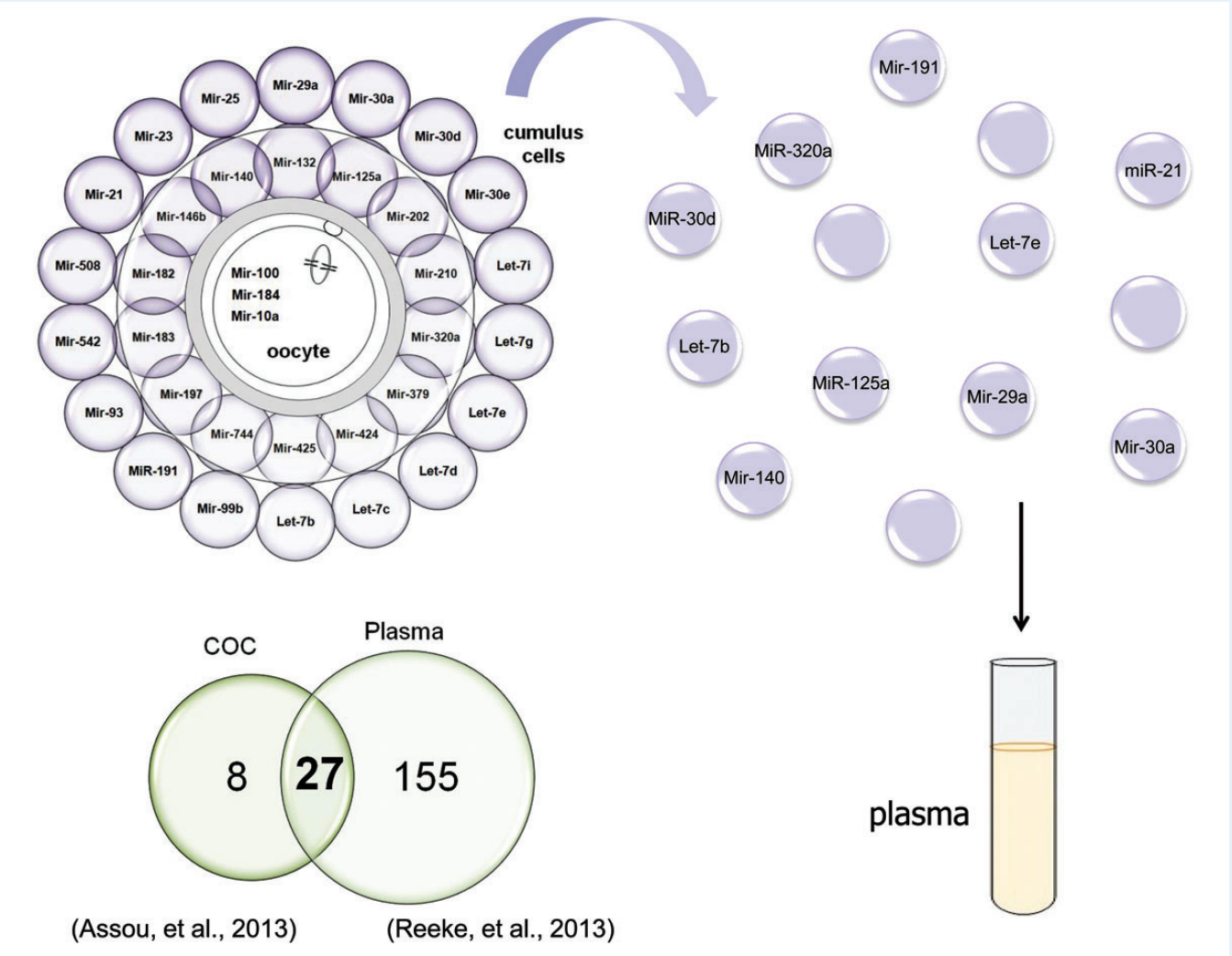

Figure 1 miRNAs expressed in human CCs and oocytes that are also present in human plasma. The Venn diagram has been generated crossing the miRNAs identified in the COC by Assou *et al.* (2013a), and the miRNAs present in plasma as described in Reeker *et al.* (2013). CC, cumulus cell.

hESCs, including miR-320, miR-92a, let-7a and miR-146b (Yang *et al.*, 2008; McCallie *et al.*, 2010; Merkerova *et al.*, 2010). Recent reports indicate that deregulated miRNA expression in the embryo is associated with human infertility (McCallie *et al.*, 2010) and the embryo miRNA expression profile varies according to its chromosomal make-up and sex (Tzur *et al.*, 2008; Rosenbluth *et al.*, 2013).

As miRNAs have been detected in the culture medium following release by cells grown in culture (Hergenreider *et al.*, 2012), it would be possible to quantify the embryonic miRNAs released in the medium in order to monitor embryo health during preimplantation *in vitro* culture. Currently, human embryo selection for transfer into the uterus is based mainly on morphology (Sakkas *et al.*, 2001; Fenwick *et al.*, 2002). The culture medium could routinely be discarded at each step of the *in vitro* embryo development. Thus, the CC gene expression profiling, together with miRNA quantification in culture medium, could improve the monitoring of preimplantation embryo health, and provide a non-invasive approach to predict oocyte competence and pregnancy outcome (Assou *et al.*, 2008, 2011, 2013b). Changes in metabolite (pyruvate, glucose or amino acids) levels in the embryo culture medium might reflect embryo viability, and these metabolites have been assessed as potential biomarkers of embryo quality (Seli *et al.*, 2007, 2010, 2011). Different methods (visual inspection, CC gene profiling, metabolite and miRNA quantification in the embryo culture medium) could thus be applied to improve embryo selection and ultimately IVF outcome.

Recently, it has been reported that the presence of cfDNA released into embryo culture medium from mitochondria is associated with poor embryo quality during cleavage (Stigliani *et al.*, 2013). Thus, as for miRNAs, the analysis of cfDNA released into the culture medium by embryos might offer the possibility to develop a non-invasive test for the selection of the embryos with the highest implantation potential.

Conclusions and further perspectives

In recent years, our understanding of the biology of circulating nucleic acids has greatly progressed and powerful technologies for their analysis

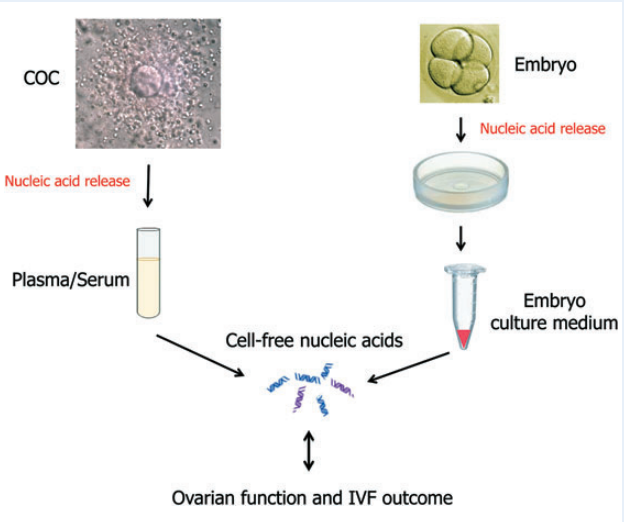

Figure 2 Model of how cell-free nucleic acids could be used as new non-invasive biomarkers of ovarian function and IVF outcome. COC, cumulus oocyte complex.

have been developed. Consequently, cell-free nucleic acids, such as cfDNA and miRNAs, will play an increasing role as non-invasive tools for the detection/prognosis of ovarian disorders and the monitoring of human preimplantation embryo health during *in vitro* culture. Indeed, blood and spent embryo culture medium are easily accessible and are therefore ideal materials for assessing ovarian and embryo health (Fig. 2). For example, the evaluation of cfDNA and miRNAs in the serum of infertile women might allow an assessment of ovarian reserve. Similarly, the quantification of cfDNA and miRNAs in micro-drops of embryo culture medium at Day 3 and Day 5/6 post-fertilization could help in the monitoring of embryo development and pregnancy outcome. Ultimately, specific embryonic miRNAs secreted into the culture medium might be attractive candidate biomarkers to predict embryo quality and pregnancy outcomes.

Authors' roles

S.T., S.A., E.S., S.B. and S.H. were involved in the study design and manuscript preparation. S.T. conducted literature review, manuscript drafting on circulating cell-free nucleic acids, and S.A. on nucleic acids in embryo culture medium and figure drafting. T.A-.E. and D.H. provided technical help and advices. S.H. provided final manuscript approbation.

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Conflict of interest

The authors declare that there is no conflict of interest as defined by the guidelines of the International Committee of Medical journal Editors (ICMJE; www.icmje.org).

References

Abd El Naby WS, Hagos TH, Hossain MM, Salilew-Wondim D, Gad AY, Rings F, Cinar MU, Tholen E, Looft C, Schellander K *et al.* Expression analysis of regulatory microRNAs in bovine cumulus oocyte complex and preimplantation embryos. *Zygote* 2013;**21**:31–51.

Adachi T, Nakanishi M, Otsuka Y, Nishimura K, Hirokawa G, Goto Y, Nonogi H, Iwai N. Plasma microRNA 499 as a biomarker of acute myocardial infarction. *Clin Chem* 2010;**56**:1183–1185.

Ai J, Zhang R, Li Y, Pu J, Lu Y, Jiao J, Li K, Yu B, Li Z, Wang R *et al.* Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. *Biochem Biophys Res Commun* 2010;**391**:73–77.

Albertini DF, Combelles CM, Benecchi E, Carabatsos MJ. Cellular basis for paracrine regulation of ovarian follicle development. *Reproduction* 2001;**121**:647–653.

Altmae S, Martinez-Conejero JA, Esteban FJ, Ruiz-Alonso M, Stavreus-Evers A, Horcajadas JA, Salumets A. MicroRNAs miR-30b, miR-30d, and miR-494 regulate human endometrial receptivity. *Reprod Sci* 2013;**20**:308–317.

Amicucci P, Gennarelli M, Novelli G, Dallapiccola B. Prenatal diagnosis of myotonic dystrophy using fetal DNA obtained from maternal plasma. *Clin Chem* 2000; **46**:301–302.

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

Asaga S, Kuo C, Nguyen T, Terpenning M, Giuliano AE, Hoon DS. Direct serum assay for microRNA-21 concentrations in early and advanced breast cancer. *Clin Chem* 2011;**57**:84–91.

Assou S, Haouzi D, Mahmoud K, Aouacheria A, Guillemin Y, Pantesco V, Reme T, Dechaud H, De Vos J, Hamamah S. A non-invasive test for assessing embryo potential by gene expression profiles of human cumulus cells: a proof of concept study. *Mol Hum Reprod* 2008;**14**:711–719.

Assou S, Haouzi D, De Vos J, Hamamah S. Human cumulus cells as biomarkers for embryo and pregnancy outcomes. *Mol Hum Reprod* 2010;**16**:531–538.

Assou S, Boumela I, Haouzi D, Anahory T, Dechaud H, De Vos J, Hamamah S. Dynamic changes in gene expression during human early embryo development: from fundamental aspects to clinical applications. *Hum Reprod Update* 2011;**17**:272–290.

Assou S, Al-edani T, Haouzi D, Philippe N, Lecellier CH, Piquemal D, Commes T, Ait-Ahmed O, Dechaud H, Hamamah S. MicroRNAs: new candidates for the regulation of the human cumulus-oocyte complex. *Hum Reprod* 2013a; **28**:3038–3049.

Assou S, Haouzi D, Dechaud H, Gala A, Ferrieres A, Hamamah S. Comparative gene expression profiling in human cumulus cells according to ovarian gonadotropin treatments. *Biomed Res Int* 2013b;**2013**:354582.

Azhar S. MicroRNA-122: a new player in the negative regulation of LH receptor expression by the LH receptor mRNA binding protein (LRBP). *Endocrinology* 2013;**154**:4439–4442.

Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab* 2004;**89**:2745–2749.

Bauer M, Hutterer G, Eder M, Majer S, Leshane E, Johnson KL, Peter I, Bianchi DW, Pertl B. A prospective analysis of cell-free fetal DNA concentration in maternal plasma as an indicator for adverse pregnancy outcome. *Prenat Diagn* 2006; **26**:831–836.

Benn P, Cuckle H, Pergament E. Non-invasive prenatal testing for aneuploidy: current status and future prospects. *Ultrasound Obstet Gynecol* 2013;**42**:15–33.

Bianchi DW. Circulating fetal DNA: its origin and diagnostic potential—a review. *Placenta* 2004;**25**(Suppl A):S93–S101.

Bianchi DW, Wilkins-Haug L. Integration of noninvasive DNA testing for aneuploidy into prenatal care: what has happened since the rubber met the road? *Clin Chem* 2014;**60**:78–87.

Bischoff FZ, Sinacori MK, Dang DD, Marquez-Do D, Horne C, Lewis DE, Simpson JL. Cell-free fetal DNA and intact fetal cells in maternal blood circulation: implications for first and second trimester non-invasive prenatal diagnosis. *Hum Reprod Update* 2002;**8**:493–500.

Bischoff FZ, Lewis DE, Simpson JL. Cell-free fetal DNA in maternal blood: kinetics, source and structure. *Hum Reprod Update* 2005;**11**:59–67.

Boon RA, Vickers KC. Intercellular transport of microRNAs. *Arterioscler Thromb Vasc Biol* 2013;**33**:186–192.

Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, Eijkemans MJ, Mol BW, Broekmans FJ. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. *Hum Reprod Update* 2013;**19**: 26–36.

Caburet S, Georges A, L'Hote D, Todeschini AL, Benayoun BA, Veitia RA. The transcription factor FOXL2: at the crossroads of ovarian physiology and pathology. *Mol Cell Endocrinol* 2012;**356**:55–64.

Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; **6**:857–866.

Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K *et al.* Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2002;**99**:15524–15529.

Canick JA, Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE. The impact of maternal plasma DNA fetal fraction on next generation sequencing tests for common fetal aneuploidies. *Prenat Diagn* 2013;**33**:667–674.

Carletti MZ, Christenson LK. MicroRNA in the ovary and female reproductive tract. *J Anim Sci* 2009;**87**:E29–E38.

Carletti MZ, Fiedler SD, Christenson LK. MicroRNA 21 blocks apoptosis in mouse periovulatory granulosa cells. *Biol Reprod* 2010;**83**:286–295.

Chaffin CL, Schwinof KM, Stouffer RL. Gonadotropin and steroid control of granulosa cell proliferation during the periovulatory interval in rhesus monkeys. *Biol Reprod* 2001;**65**:755–762.

Chan M, Liaw CS, Ji SM, Tan HH, Wong CY, Thike AA, Tan PH, Ho GH, Lee AS. Identification of circulating microRNA signatures for breast cancer detection. *Clin Cancer Res* 2013;**19**:4477–4487.

Chen ZB, Yan M, Lei Z, Xiao B, Liang Y, Zhu ZL, Huang LJ, Liu JZ. Application of fetal DNA in maternal plasma for non-invasive prenatal diagnosis of fetal sex. *Yi Chuan* 2004;**26**:18–22.

Chen X, Ren JH, Guo H, Lin LH, Yao QX. Cell-free fetal DNA detection in maternal plasma using real-time PCR and cycling probe technology for prenatal screening beta-thalassaemia major. *Nan Fang Yi Ke Da Xue Xue Bao* 2008; **28**:1210–1213.

Chen YH, Heneidi S, Lee JM, Layman LC, Stepp DW, Gamboa GM, Chen BS, Chazenbalk G, Azziz R. miRNA-93 inhibits GLUT4 and is overexpressed in adipose tissue of polycystic ovary syndrome patients and women with insulin resistance. *Diabetes* 2013;**62**:2278–2286.

Chim SS, Shing TK, Hung EC, Leung TY, Lau TK, Chiu RW, Lo YM. Detection and characterization of placental microRNAs in maternal plasma. *Clin Chem* 2008; **54**:482–490.

Chiu RW, Lau TK, Cheung PT, Gong ZQ, Leung TN, Lo YM. Noninvasive prenatal exclusion of congenital adrenal hyperplasia by maternal plasma analysis: a feasibility study. *Clin Chem* 2002a;**48**:778–780.

Chiu RW, Lau TK, Leung TN, Chow KC, Chui DH, Lo YM. Prenatal exclusion of beta thalassaemia major by examination of maternal plasma. *Lancet* 2002b;**360**: 998–1000.

Cho WC. OncomiRs: the discovery and progress of microRNAs in cancers. *Mol Cancer* 2007;**6**:60.

Choi WY, Giraldez AJ, Schier AF. Target protectors reveal dampening and balancing of nodal agonist and antagonist by miR-430. *Science* 2007a;**318**:271–274.

Choi Y, Qin Y, Berger MF, Ballow DJ, Bulyk ML, Rajkovic A. Microarray analyses of newborn mouse ovaries lacking Nobox. *Biol Reprod* 2007b; **77**:312–319.

Chou JS, Jacobson JD, Patton WC, King A, Chan PJ. Modified isocratic capillary electrophoresis detection of cell-free DNA in semen. *J Assist Reprod Genet* 2004; **21**:397–400.

Conn PM, Crowley WF Jr. Gonadotropin-releasing hormone and its analogs. *Annu Rev Med* 1994;**45**:391–405.

Costa JM, Benachi A, Gautier E, Jouannic JM, Ernault P, Dumez Y. First-trimester fetal sex determination in maternal serum using real-time PCR. *Prenat Diagn* 2001; **21**:1070–1074.

Costa JM, Benachi A, Gautier E, Jouannic JM, Ernault P, Dumez Y. First trimester fetal sex determination in maternal serum using real-time PCR. *Gynecol Obstet Fertil* 2002; **30**:953–957.

Cotter AM, Martin CM, O'Leary JJ, Daly SF. Increased fetal DNA in the maternal circulation in early pregnancy is associated with an increased risk of preeclampsia. *Am J Obstet Gynecol* 2004;**191**:515–520.

Cui XS, Sun SC, Kang YK, Kim NH. Involvement of microRNA-335-5p in cytoskeleton dynamics in mouse oocytes. *Reprod Fertil Dev* 2013;**25**:691–699.

Cuk K, Zucknick M, Heil J, Madhavan D, Schott S, Turchinovich A, Arlt D, Rath M, Sohn C, Benner A *et al.* Circulating microRNAs in plasma as early detection markers for breast cancer. *Int J Cancer* 2013a;**132**:1602–1612.

Cuk K, Zucknick M, Madhavan D, Schott S, Golatta M, Heil J, Marme F, Turchinovich A, Sinn P, Sohn C *et al.* Plasma microRNA panel for minimally invasive detection of breast cancer. *PLoS ONE* 2013b;**8**:e76729.

Czamanski-Cohen J, Sarid O, Cwikel J, Lunenfeld E, Douvdevani A, Levitas E, Har-Vardi I. Increased plasma cell-free DNA is associated with low pregnancy rates among women undergoing IVF-embryo transfer. *Reprod Biomed Online* 2013; **26**:36–41.

Czamanski-Cohen J, Sarid O, Cwikel J, Levitas E, Lunenfeld E, Douvdevani A, Har-Vardi I. Decrease in cell free DNA levels following participation in stress reduction techniques among women undergoing infertility treatment. *Arch Womens Ment Health* 2014;**17**:251–3.

Dai A, Sun H, Fang T, Zhang Q, Wu S, Jiang Y, Ding L, Yan G, Hu Y. MicroRNA-133b stimulates ovarian estradiol synthesis by targeting Foxl2. *FEBS Lett* 2013; **587**:2474–2482.

Dan S, Wang W, Ren J, Li Y, Hu H, Xu Z, Lau TK, Xie J, Zhao W, Huang H *et al.* Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11,105 pregnancies with mixed risk factors. *Prenat Diagn* 2012;**32**:1225–1232.

da Silveira JC, Veeramachaneni DN, Winger QA, Carnevale EM, Bouma GJ. Cell-secreted vesicles in equine ovarian follicular fluid contain miRNAs and proteins: a possible new form of cell communication within the ovarian follicle. *Biol Reprod* 2012;**86**:71.

Davalos A, Goedeke L, Smibert P, Ramirez CM, Warrier NP, Andreo U, Cirera-Salinas D, Rayner K, Suresh U, Pastor-Pareja JC *et al.* miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. *Proc Natl Acad Sci USA* 2011;**108**:9232–9237.

Deng ZH, Li DC. Progress of research on biochemistry characteristics of cell-free fetal DNA in maternal plasma and its application. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2007;**24**:314–318.

Diehl F, Li M, Dressman D, He Y, Shen D, Szabo S, Diaz LA, Goodman SN, David KA, Juhl H *et al.* Detection and quantification of mutations in the plasma of patients with colorectal tumors. *Proc Natl Acad Sci USA* 2005;**102**:16368–16373.

Dillhoff M, Liu J, Frankel W, Croce C, Bloomston M. MicroRNA-21 is overexpressed in pancreatic cancer and a potential predictor of survival. *J Gastrointest Surg* 2008; **12**:2171–2176.

Dobrzycka B, Terlikowski SJ, Mazurek A, Kowalczuk O, Niklinska W, Chyczewski L, Kulikowski M. Circulating free DNA, p53 antibody and mutations of KRAS gene in endometrial cancer. *Int J Cancer* 2010;**127**:612–621.

Dobrzycka B, Terlikowski SJ, Kinalski M, Kowalczuk O, Niklinska W, Chyczewski L. Circulating free DNA and p53 antibodies in plasma of patients with ovarian epithelial cancers. *Ann Oncol* 2011;**22**:1133–1140.

Eichelser C, Flesch-Janys D, Chang-Claude J, Pantel K, Schwarzenbach H. Deregulated serum concentrations of circulating cell-free microRNAs miR-17, miR-34a, miR-155, and miR-373 in human breast cancer development and progression. *Clin Chem* 2013;**59**:1489–1496.

Eppig J. Mouse oocytes control metabolic co-operativity between oocytes and cumulus cells. *Reprod Fertil Dev* 2005;**17**:1–2.

Esau CC, Monia BP. Therapeutic potential for microRNAs. *Adv Drug Deliv Rev* 2007; **59**:101–114.

Esquela-Kerscher A, Slack FJ. Oncomirs—microRNAs with a role in cancer. *Nat Rev Cancer* 2006;**6**:259–269.

Fairbrother G, Johnson S, Musci TJ, Song K. Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18, and 13, in a general screening population. *Prenat Diagn* 2013;**33**:580–583.

Farina A, LeShane ES, Lambert-Messerlian GM, Canick JA, Lee T, Neveux LM, Palomaki GE, Bianchi DW. Evaluation of cell-free fetal DNA as a second-trimester maternal serum marker of Down syndrome pregnancy. *Clin Chem* 2003;**49**: 239–242.

Farr RJ, Joglekar MV, Taylor CJ, Hardikar AA. Circulating non-coding RNAs as biomarkers of beta cell death in diabetes. *Pediatr Endocrinol Rev* 2013;**11**:14–20.

Fenwick J, Platteau P, Murdoch AP, Herbert M. Time from insemination to first cleavage predicts developmental competence of human preimplantation embryos *in vitro*. *Hum Reprod* 2002;**17**:407–412.

Fernandez-Valverde SL, Taft RJ, Mattick JS. MicroRNAs in beta-cell biology, insulin resistance, diabetes and its complications. *Diabetes* 2011;**60**:1825–1831.

Fiedler SD, Carletti MZ, Hong X, Christenson LK. Hormonal regulation of microRNA expression in periovulatory mouse mural granulosa cells. *Biol Reprod* 2008;**79**: 1030–1037.

Fiore R, Schratt G. MicroRNAs in vertebrate synapse development. *ScientificWorldJournal* 2007;**7**:167–177.

Foshay KM, Gallicano GI. miR-17 family miRNAs are expressed during early mammalian development and regulate stem cell differentiation. *Dev Biol* 2009; **326**:431–443.

Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 2009;**19**:92–105.

Gahan PB, Anker P, Stroun M. Metabolic DNA as the origin of spontaneously released DNA? *Ann N Y Acad Sci* 2008;**1137**:7–17.

Ge H, Huang X, Li X, Chen S, Zheng J, Jiang H, Zhang C, Pan X, Guo J, Chen F *et al.* Noninvasive prenatal detection for pathogenic CNVs: the application in alpha-thalassemia. *PLoS ONE* 2013;**8**:e67464.

Gebremedhn S, Ahmad I, Salilew-Wondim D, Sahadevan S, Hoelker M, Rings F, Udin J, Tholen E, Looft C, Schellander K *et al.* 113 expression profiling of noncoding microRNAs in bovine granulosa cells of preovulatory dominant follicle using deep sequencing. *Reprod Fertil Dev* 2013;**26**:170–171.

Gerin I, Clerbaux LA, Haumont O, Lanthier N, Das AK, Burant CF, Leclercq IA, MacDougald OA, Bommer GT. Expression of miR-33 from an SREBP2 intron inhibits cholesterol export and fatty acid oxidation. *J Biol Chem* 2010; **285**:33652–33661.

Gilabert-Estelles J, Braza-Boils A, Ramon LA, Zorio E, Medina P, Espana F, Estelles A. Role of microRNAs in gynecological pathology. *Curr Med Chem* 2012; **19**:2406–2413.

Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. *Hum Reprod Update* 2008;**14**:159–177.

Godoy J, Nishimura M, Webster NJ. Gonadotropin-releasing hormone induces miR-132 and miR-212 to regulate cellular morphology and migration in immortalized LbetaT2 pituitary gonadotrope cells. *Mol Endocrinol* 2011; **25**:810–820.

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

Gonzalez-Gonzalez MC, Garcia-Hoyos M, Trujillo MJ, Rodriguez de Alba M, Lorda-Sanchez I, Diaz-Recasens J, Gallardo E, Ayuso C, Ramos C. Prenatal detection of a cystic fibrosis mutation in fetal DNA from maternal plasma. *Prenat Diagn* 2002;**22**:946–948.

Gonzalez-Gonzalez MC, Trujillo MJ, Rodriguez de Alba M, Ramos C. Early Huntington disease prenatal diagnosis by maternal semiquantitative fluorescent-PCR. *Neurology* 2003;**60**:1214–1215.

Gonzalez-Gonzalez C, Garcia-Hoyos M, Trujillo-Tiebas MJ, Lorda-Sanchez I, de Alba MR, Infantes F, Gallego J, Diaz-Recasens J, Ayuso C, Ramos C. Application of fetal DNA detection in maternal plasma: a prenatal diagnosis unit experience. *J Histochem Cytochem* 2005;**53**:307–314.

Gorzelnik K1, Bijok J, Zimowski JG, Jakiel G, Roszkowski T. Noninvasive prenatal diagnosis of trisomy 21, 18 and 13 using cell-free fetal DNA. *Ginekol Pol* 2013; **84**:714–719.

Gunel T, Zeybek YG, Akcakaya P, Kalelioglu I, Benian A, Ermis H, Aydinli K. Serum microRNA expression in pregnancies with preeclampsia. *Genet Mol Res* 2011; **10**:4034–4040.

Guo LJ, Zhang QY. Decreased serum miR-181a is a potential new tool for breast cancer screening. *Int J Mol Med* 2012;**30**:680–686.

Guo F, Tian J, Lin Y, Jin Y, Wang L, Cui M. Serum microRNA-92 expression in patients with ovarian epithelial carcinoma. *J Int Med Res* 2013;**41**:1456–1461.

Haghiac M, Vora NL, Basu S, Johnson KL, Presley L, Bianchi DW, Hauguel-de Mouzon S. Increased death of adipose cells, a path to release cell-free DNA into systemic circulation of obese women. *Obesity (Silver Spring)* 2012;**20**:2213–2219.

Hahn S, Holzgreve W. Fetal cells and cell-free fetal DNA in maternal blood: new insights into pre-eclampsia. *Hum Reprod Update* 2002;**8**:501–508.

Hahn S, Huppertz B, Holzgreve W. Fetal cells and cell free fetal nucleic acids in maternal blood: new tools to study abnormal placentation? *Placenta* 2005;**26**: 515–526.

Hahn S, Rusterholz C, Hosli I, Lapaire O. Cell-free nucleic acids as potential markers for preeclampsia. *Placenta* 2011;**32**(Suppl):S17–S20.

Hansen T, Olsen L, Lindow M, Jakobsen KD, Ullum H, Jonsson E, Andreassen OA, Djurovic S, Melle I, Agartz I *et al.* Brain expressed microRNAs implicated in schizophrenia etiology. *PLoS ONE* 2007;**2**:e873.

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.

Hawkins SM, Matzuk MM. Oocyte-somatic cell communication and microRNA function in the ovary. *Ann Endocrinol (Paris)* 2010;**71**:144–148.

Heneghan HM, Miller N, Kelly R, Newell J, Kerin MJ. Systemic miRNA-195 differentiates breast cancer from other malignancies and is a potential biomarker for detecting noninvasive and early stage disease. *Oncologist* 2010;**15**:673–682.

Hergenreider E, Heydt S, Treguer K, Boettger T, Horrevoets AJ, Zeiher AM, Scheffer MP, Frangakis AS, Yin X, Mayr M *et al.* Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nat Cell Biol* 2012;**14**:249–256.

Hill M, Lewis C, Jenkins L, Allen S, Elles RG, Chitty LS. Implementing noninvasive prenatal fetal sex determination using cell-free fetal DNA in the United Kingdom. *Expert Opin Biol Ther* 2012;**12**(Suppl 1):S119–S126.

Holdenrieder S, Stieber P, Bodenmuller H, Busch M, Von Pawel J, Schalhorn A, Nagel D, Seidel D. Circulating nucleosomes in serum. *Ann N Y Acad Sci* 2001a;**945**:93–102.

Holdenrieder S, Stieber P, Bodenmuller H, Fertig G, Furst H, Schmeller N, Untch M, Seidel D. Nucleosomes in serum as a marker for cell death. *Clin Chem Lab Med* 2001b;**39**:596–605.

Honda H, Miharu N, Ohashi Y, Samura O, Kinutani M, Hara T, Ohama K. Fetal gender determination in early pregnancy through qualitative and quantitative analysis of fetal DNA in maternal serum. *Hum Genet* 2002;**110**:75–79.

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.

Hong F, Li Y, Xu Y, Zhu L. Prognostic significance of serum microRNA-221 expression in human epithelial ovarian cancer. *J Int Med Res* 2013;**41**:64–71.

Horie T, Ono K, Horiguchi M, Nishi H, Nakamura T, Nagao K, Kinoshita M, Kuwabara Y, Marusawa H, Iwanaga Y *et al.* MicroRNA-33 encoded by an intron of sterol regulatory element-binding protein 2 (Srebp2) regulates HDL *in vivo*. *Proc Natl Acad Sci USA* 2010;**107**:17321–17326.

Horsting JM, Dlouhy SR, Hanson K, Quaid K, Bai S, Hines KA. Genetic counselors' experience with cell-free fetal DNA testing as a prenatal screening option for aneuploidy. *J Genet Couns* 2014;**23**:377–400.

Hossain MM, Sohel MM, Schellander K, Tesfaye D. Characterization and importance of microRNAs in mammalian gonadal functions. *Cell Tissue Res* 2012;**349**:679–690.

Hou QF, Wu D, Chu Y, Kang B, Liao SX, Yang YL, Zhang CY, Zhang JX, Wu G. Clinical application of noninvasive prenatal diagnosis using cell free fetal DNA in maternal plasma. *Zhonghua Fu Chan Ke Za Zhi* 2012;**47**:813–817.

Hui L, Bianchi DW. Recent advances in the prenatal interrogation of the human fetal genome. *Trends Genet* 2013;**29**:84–91.

Hulsmans M, Holvoet P. MicroRNAs as early biomarkers in obesity and related metabolic and cardiovascular diseases. *Curr Pharm Des.* 2013;**19**:5704–5717.

Hulsmans M, De Keyzer D, Holvoet P. MicroRNAs regulating oxidative stress and inflammation in relation to obesity and atherosclerosis. *FASEB J* 2011; **25**:2515–2527.

Huppertz B, Kingdom JC. Apoptosis in the trophoblast—role of apoptosis in placental morphogenesis. *J Soc Gynecol Investig* 2004;**11**:353–362.

Hutvagner G, Simard MJ. Argonaute proteins: key players in RNA silencing. *Nat Rev Mol Cell Biol* 2008;**9**:22–32.

Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 2001;**293**:834–838.

Illanes S, Soothill P. Noninvasive approach for the management of hemolytic disease of the fetus. *Expert Rev Hematol.* 2009;**2**:577–582.

Jia SZ, Yang Y, Lang J, Sun P, Leng J. Plasma miR-17–5p, miR-20a and miR-22 are down-regulated in women with endometriosis. *Hum Reprod* 2013;**28**: 322–330.

Kaiser UB, Conn PM, Chin WW. Studies of gonadotropin-releasing hormone (GnRH) action using GnRH receptor-expressing pituitary cell lines. *Endocr Rev* 1997; **18**:46–70.

Kamat AA, Bischoff FZ, Dang D, Baldwin MF, Han LY, Lin YG, Merritt WM, Landen CN Jr, Lu C, Gershenson DM *et al.* Circulating cell-free DNA: a novel biomarker for response to therapy in ovarian carcinoma. *Cancer Biol Ther* 2006a;**5**:1369–1374.

Kamat AA, Sood AK, Dang D, Gershenson DM, Simpson JL, Bischoff FZ. Quantification of total plasma cell-free DNA in ovarian cancer using real-time PCR. *Ann N Y Acad Sci* 2006b;**1075**:230–234.

Kamat AA, Baldwin M, Urbauer D, Dang D, Han LY, Godwin A, Karlan BY, Simpson JL, Gershenson DM, Coleman RL *et al.* Plasma cell-free DNA in ovarian cancer: an independent prognostic biomarker. *Cancer* 2010;**116**:1918–1925.

Kan CW, Hahn MA, Gard GB, Maidens J, Huh JY, Marsh DJ, Howell VM. Elevated levels of circulating microRNA-200 family members correlate with serous epithelial ovarian cancer. *BMC Cancer* 2012;**12**:627.

Khorram Khorshid HR, Zargari M, Sadeghi MR, Edallatkhah H, Shahhosseiny MH, Kamali K. Early fetal gender determination using real-time PCR analysis of cell-free fetal DNA during 6th-10th weeks of gestation. *Acta Med Iran* 2013; **51**:209–214.

Kim YJ, Ku SY, Kim YY, Liu HC, Chi SW, Kim SH, Choi YM, Kim JG, Moon SY. MicroRNAs transfected into granulosa cells may regulate oocyte meiotic competence during *in vitro* maturation of mouse follicles. *Hum Reprod* 2013; **28**:3050–3061.

Kumar S, Keerthana R, Pazhanimuthu A, Perumal P. Overexpression of circulating miRNA-21 and miRNA-146a in plasma samples of breast cancer patients. *Indian J Biochem Biophys* 2013;**50**:210–214.

Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. *Science* 2001;**294**:853–858.

Laktionov PP, Tamkovich SN, Rykova EY, Bryzgunova OE, Starikov AV, Kuznetsova NP, Sumarokov SV, Kolomiets SA, Sevostianova NV, Vlassov VV. Extracellular circulating nucleic acids in human plasma in health and disease. *Nucleosides Nucleotides Nucleic Acids* 2004;**23**:879–883.

Lambert-Messerlian G, Kloza EM, Williams J III, Loucky J, O'Brien B, Wilkins-Haug L, Mahoney MJ, De Biasio P, Borrell A, Ehrich M *et al.* Maternal plasma DNA testing for aneuploidy in pregnancies achieved by assisted reproductive technologies. *Genet Med* 2014;**16**:419–422.

Langlois S, Brock JA, Wilson RD, Audibert F, Carroll J, Cartier L, Gagnon A, Johnson JA, Macdonald W, Murphy-Kaulbeck L *et al.* Current status in non-invasive prenatal detection of down syndrome, trisomy 18, and trisomy 13 using cell-free DNA in maternal plasma. *J Obstet Gynaecol Can* 2013;**35**:177–181.

Lapaire O, Volgmann T, Grill S, Hosli I, Zanetti-Daellenbach R, Zhong XY, Holzgreve W. Significant correlation between maternal body mass index at delivery and in the second trimester, and second trimester circulating total cell-free DNA levels. *Reprod Sci* 2009;**16**:274–279.

Latronico MV, Catalucci D, Condorelli G. Emerging role of microRNAs in cardiovascular biology. *Circ Res* 2007;**101**:1225–1236.

Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultwood J, Wainscoat JS *et al.* Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haematol* 2008;**141**:672–675.

Lazar L, Rigo J Jr, Nagy B, Balogh K, Mako V, Cervenak L, Mezes M, Prohaszka Z, Molvarec A. Relationship of circulating cell-free DNA levels to cell-free fetal DNA levels, clinical characteristics and laboratory parameters in preeclampsia. *BMC Med Genet* 2009;**10**:120.

Lazar L, Nagy B, Molvarec A, Rigo J Jr. Quantity of total cell free and cell free fetal DNA in pregnancies with no complications and with preeclampsia. *Orv Hetil* 2010; **151**:784–787.

Lazar DA, Cohen SA, Evora DK, Losasso BE, Bickler SW. Cecal bascule in a child: an unusual cause of postoperative bowel obstruction. *J Pediatr Surg* 2012;**47**:609–611.

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.

Lee T, LeShane ES, Messerlian GM, Canick JA, Farina A, Heber WW, Bianchi DW. Down syndrome and cell-free fetal DNA in archived maternal serum. *Am J Obstet Gynecol* 2002;**187**:1217–1221.

Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* 2004;**23**:4051–4060.

Lei L, Jin S, Gonzalez G, Behringer RR, Woodruff TK. The regulatory role of Dicer in folliculogenesis in mice. *Mol Cell Endocrinol* 2010;**315**:63–73.

Leung TN, Zhang J, Lau TK, Hjelm NM, Lo YM. Maternal plasma fetal DNA as a marker for preterm labour. *Lancet* 1998;**352**:1904–5.

Leung TN, Zhang J, Lau TK, Chan LY, Lo YM. Increased maternal plasma fetal DNA concentrations in women who eventually develop preeclampsia. *Clin Chem* 2001; **47**:137–139.

Levine RJ, Hauth JC, Curet LB, Sibai BM, Catalano PM, Morris CD, DerSimonian R, Esterlitz JR, Raymond EG, Bild DE *et al.* Trial of calcium to prevent preeclampsia. *N Engl J Med* 1997;**337**:69–76.

Levine RJ, Qian C, Leshane ES, Yu KF, England LJ, Schisterman EF, Wataganara T, Romero R, Bianchi DW. Two-stage elevation of cell-free fetal DNA in maternal sera before onset of preeclampsia. *Am J Obstet Gynecol* 2004;**190**:707–713.

Li GH, Rong KB, Luo YF, Chen D, Gong CP, Wu J, Di YW, Ge YF. Prenatal diagnosis of beta-thalassaemia using cell-free fetal DNA in maternal plasma. *Nan Fang Yi Ke Da Xue Xue Bao* 2011;**31**:1437–1439.

Liggett TE, Melnikov A, Yi Q, Replogle C, Hu W, Rotmensch J, Kamat A, Sood AK, Levenson V. Distinctive DNA methylation patterns of cell-free plasma DNA in women with malignant ovarian tumors. *Gynecol Oncol* 2011a;**120**:113–120.

Liggett TE, Melnikov AA, Marks JR, Levenson VV. Methylation patterns in cell-free plasma DNA reflect removal of the primary tumor and drug treatment of breast cancer patients. *Int J Cancer* 2011b;**128**:492–499.

Lim JH, Kim MJ, Kim SY, Kim HO, Song MJ, Kim MH, Park SY, Yang JH, Ryu HM. Non-invasive prenatal detection of achondroplasia using circulating fetal DNA in maternal plasma. *J Assist Reprod Genet* 2011;**28**:167–172.

Lin F, Li R, Pan ZX, Zhou B, Yu de B, Wang XG, Ma XS, Han J, Shen M, Liu HL. miR-26b promotes granulosa cell apoptosis by targeting ATM during follicular atresia in porcine ovary. *PLoS ONE* 2012;**7**:e38640.

Liu J, Mao Q, Liu Y, Hao X, Zhang S, Zhang J. Analysis of miR-205 and miR-155 expression in the blood of breast cancer patients. *Chin J Cancer Res* 2013;**25**:46–54.

Lo YM. Recent advances in fetal nucleic acids in maternal plasma. *J Histochem Cytochem* 2005;**53**:293–296.

Lo YM, Chiu RW. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidies by maternal plasma nucleic acid analysis. *Clin Chem* 2008;**54**:461–466.

Lo YM, Lau TK, Zhang J, Leung TN, Chang AM, Hjelm NM, Elmes RS, Bianchi DW. Increased fetal DNA concentrations in the plasma of pregnant women carrying fetuses with trisomy 21. *Clin Chem* 1999;**45**:1747–1751.

Ma T, Jiang H, Gao Y, Zhao Y, Dai L, Xiong Q, Xu Y, Zhao Z, Zhang J. Microarray analysis of differentially expressed microRNAs in non-regressed and regressed bovine corpus luteum tissue;microRNA-378 may suppress luteal cell apoptosis by targeting the interferon gamma receptor 1 gene. *J Appl Genet* 2011;**52**:481–486.

Mar-Aguilar F, Mendoza-Ramirez JA, Malagon-Santiago I, Espino-Silva PK, Santuario-Facio SK, Ruiz-Flores P, Rodriguez-Padilla C, Resendez-Perez D. Serum circulating microRNA profiling for identification of potential breast cancer biomarkers. *Dis Markers* 2013;**34**:163–169.

Marquart TJ, Allen RM, Ory DS, Baldan A. miR-33 links SREBP-2 induction to repression of sterol transporters. *Proc Natl Acad Sci USA* 2010;**107**:12228–12232.

Mase Y, Ishibashi O, Ishikawa T, Takizawa T, Kiguchi K, Ohba T, Katabuchi H, Takeshita T. MiR-21 is enriched in the RNA-induced silencing complex and targets COL4A1 in human granulosa cell lines. *Reprod Sci* 2012;**19**:1030–1040.

McBride D, Carre 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.

McCallie B, Schoolcraft WB, Katz-Jaffe MG. Aberration of blastocyst microRNA expression is associated with human infertility. *Fertil Steril* 2010;**93**:2374–2382.

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 USA* 2011; **108**:14163–14168.

Menon B, Sinden J, Franzo-Romain M, Botta RB, Menon KM. Regulation of LH receptor mRNA binding protein by miR-122 in rat ovaries. *Endocrinology* 2013; **154**:4826–4834.

Merkerova M, Vasikova A, Belickova M, Bruchova H. MicroRNA expression profiles in umbilical cord blood cell lineages. *Stem Cells Dev* 2010;**19**:17–26.

Miles JR, McDaneld TG, Wiedmann RT, Cushman RA, Echternkamp SE, Vallet JL, Smith TP. MicroRNA expression profile in bovine cumulus-oocyte complexes: possible role of let-7 and miR-106a in the development of bovine oocytes. *Anim Reprod Sci* 2012;**130**:16–26.

Miranda ML, Macher HC, Munoz-Hernandez R, Vallejo-Vaz A, Moreno-Luna R, Villar J, Guerrero JM, Stiefel P. Role of circulating cell-free DNA levels in patients with severe preeclampsia and HELLP syndrome. *Am J Hypertens* 2013;**26**:1377–1380.

Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A *et al.* Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008; **105**:10513–10518.

Miura K, Higashijima A, Shimada T, Miura S, Yamasaki K, Abe S, Jo O, Kinoshita A, Yoshida A, Yoshimura S *et al.* Clinical application of fetal sex determination using cell-free fetal DNA in pregnant carriers of X-linked genetic disorders. *J Hum Genet* 2011;**56**:296–299.

Montagnana M, Lippi G, Guidi GC. New frontiers in noninvasive, prenatal screening and diagnosis: fetal DNA circulating in maternal blood. *Minerva Ginecol* 2007; **59**:331–341.

Mori T, Amano T, Shimizu H. Roles of gap junctional communication of cumulus cells in cytoplasmic maturation of porcine oocytes cultured *in vitro*. *Biol Reprod* 2000; **62**:913–919.

Murchison EP, Stein P, Xuan Z, Pan H, Zhang MQ, Schultz RM, Hannon GJ. Critical roles for Dicer in the female germline. *Genes Dev* 2007;**21**:682–693.

Murri M, Insenser M, Fernandez-Duran E, San-Millan JL, Escobar-Morreale HF. Effects of polycystic ovary syndrome (PCOS), sex hormones, and obesity on circulating miRNA-21, miRNA-27b, miRNA-103, and miRNA-155 expression. *J Clin Endocrinol Metab* 2013;**98**:E1835–E1844.

Murtaza M, Dawson SJ, Tsui DW, Gale D, Forshew T, Piskorz AM, Parkinson C, Chin SF, Kingsbury Z, Wong AS *et al.* Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* 2013;**497**:108–112.

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.

Najafi-Shoushtari SH, Kristo F, Li Y, Shioda T, Cohen DE, Gerszten RE, Naar AM. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. *Science* 2010;**328**:1566–1569.

Ng EK, Li R, Shin VY, Jin HC, Leung CP, Ma ES, Pang R, Chua D, Chu KM, Law WL *et al.* Circulating microRNAs as specific biomarkers for breast cancer detection. *PLoS ONE* 2013;**8**:e53141.

Nicolaides KH, Syngelaki A, Poon LC, Gil MM, Wright D. First-trimester contingent screening for trisomies 21, 18 and 13 by biomarkers and maternal blood cell-free DNA testing. *Fetal Diagn Ther* 2014;**35**:118–126.

No JH, Kim K, Park KH, Kim YB. Cell-free DNA level as a prognostic biomarker for epithelial ovarian cancer. *Anticancer Res* 2012;**32**:3467–3471.

Norton ME, Brar H, Weiss J, Karimi A, Laurent LC, Caughey AB, Rodriguez MH, Williams J III, Mitchell ME, Adair CD *et al.* Non-Invasive Chromosomal Evaluation (NICE) study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012;**207**:137 e131–138.

Ohashi Y, Miharu N, Honda H, Samura O, Ohama K. Quantitation of fetal DNA in maternal serum in normal and aneuploid prenancies. *Hum Genet* 2001;**108**:123–127.

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

Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Grody WW *et al.* DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med* 2012;**14**:296–305.

Pangas SA, Choi Y, Ballow DJ, Zhao Y, Westphal H, Matzuk MM, Rajkovic A. Oogenesis requires germ cell-specific transcriptional regulators Sohlh1 and Lhx8. *Proc Natl Acad Sci USA* 2006;**103**:8090–8095.

Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. *Endocr Rev* 1997;**18**:361–377.

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.

Perkins DO, Jeffries CD, Jarskog LF, Thomson JM, Woods K, Newman MA, Parker JS, Jin J, Hammond SM. microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. *Genome Biol* 2007;**8**:R27.

Pescador N, Perez-Barba M, Ibarra JM, Corbaton A, Martinez-Larrad MT, Serrano-Rios M. Serum circulating microRNA profiling for identification of potential type 2 diabetes and obesity biomarkers. *PLoS ONE* 2013;**8**:e77251.

Pisarska MD, Barlow G, Kuo FT. Minireview: roles of the forkhead transcription factor FOXL2 in granulosa cell biology and pathology. *Endocrinology* 2011;**152**: 1199–1208.

Pisetsky DS, Fairhurst AM. The origin of extracellular DNA during the clearance of dead and dying cells. *Autoimmunity* 2007;**40**:281–284.

Reedy AM, Zheng Y, Jagadeeswaran G, Macmil SL, Graham WB, Roe BA, Desilva U, Zhang W, Sunkar R. Cloning, characterization and expression analysis of porcine microRNAs. *BMC Genomics* 2009;**10**:65.

Rekker K, Saare M, Roost AM, Salumets A, Peters M. Circulating microRNA profile throughout the menstrual cycle. *PLoS ONE* 2013;**8**:e81166.

Rijnders RJ, van der Schoot CE, Bossers B, de Vroede MA, Christiaens GC. Fetal sex determination from maternal plasma in pregnancies at risk for congenital adrenal hyperplasia. *Obstet Gynecol* 2001;**98**:374–378.

Roberts AC, Porter KE. Cellular and molecular mechanisms of endothelial dysfunction in diabetes. *Diab Vasc Dis Res* 2013;**10**:472–482.

Robinson C, van den Boom D, Bombard AT. Noninvasive prenatal detection of aneuploidy. *Clin Obstet Gynecol* 2014;**57**:210–25.

Robker RL, Richards JS. Hormone-induced proliferation and differentiation of granulosa cells: a coordinated balance of the cell cycle regulators cyclin D2 and p27Kip1. *Mol Endocrinol* 1998;**12**:924–940.

Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. *Genome Res* 2004; **14**:1902–1910.

Rosenbluth EM, Shelton DN, Sparks AE, Devor E, Christenson L, Van Voorhis BJ. MicroRNA expression in the human blastocyst. *Fertil Steril* 2013;**99**:855–861. e3.

Rottiers V, Naar AM. MicroRNAs in metabolism and metabolic disorders. *Nat Rev Mol Cell Biol* 2012;**13**:239–250.

Russo ML, Blakemore KJ. A historical and practical review of first trimester aneuploidy screening. *Semin Fetal Neonatal Med* 2014;**19**:183–187.

Saito H, Sekizawa A, Morimoto T, Suzuki M, Yanaihara T. Prenatal DNA diagnosis of a single-gene disorder from maternal plasma. *Lancet* 2000;**356**:1170.

Sakkas D, Percival G, D'Arcy Y, Sharif K, Afnan M. Assessment of early cleaving *in vitro* fertilized human embryos at the 2-cell stage before transfer improves embryo selection. *Fertil Steril* 2001;**76**:1150–1156.

Sang Q, Yao Z, Wang H, Feng R, Zhao X, Xing Q, Jin L, He L, Wu L, Wang L. Identification of microRNAs in human follicular fluid: characterization of microRNAs that govern steroidogenesis *in vitro* and are associated with polycystic ovary syndrome *in vivo*. *J Clin Endocrinol Metab* 2013;**98**:3068–3079.

Schmidt D, Ovitt CE, Anlag K, Fehsenfeld S, Gredsted L, Treier AC, Treier M. The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. *Development* 2004;**131**:933–942.

Schrauder MG, Strick R, Schulz-Wendtland R, Strissel PL, Kahmann L, Loehberg CR, Lux MP, Jud SM, Hartmann A, Hein A *et al.* Circulating micro-RNAs as potential blood-based markers for early stage breast cancer detection. *PLoS ONE* 2012; **7**:e29770.

Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer* 2011;**11**:426–437.

Schwarzenbach H, Milde-Langosch K, Steinbach B, Muller V, Pantel K. Diagnostic potential of PTEN-targeting miR-214 in the blood of breast cancer patients. *Breast Cancer Res Treat* 2012;**134**:933–941.

Sekizawa A, Saito H. Prenatal screening of single-gene disorders from maternal blood. *Am J Pharmacogenomics*. 2001;**1**:111–7.

Sekizawa A, Jimbo M, Saito H, Iwasaki M, Sugito Y, Yukimoto Y, Otsuka J, Okai T. Increased cell-free fetal DNA in plasma of two women with invasive placenta. *Clin Chem* 2002;**48**:353–354.

Sekizawa A, Farina A, Koide K, Iwasaki M, Honma S, Ichizuka K, Saito H, Okai T. beta-globin DNA in maternal plasma as a molecular marker of pre-eclampsia. *Prenat Diagn* 2004;**24**:697–700.

Seli E, Sakkas D, Scott R, Kwok SC, Rosendahl SM, Burns DH. Noninvasive metabolomic profiling of embryo culture media using Raman and near-infrared spectroscopy correlates with reproductive potential of embryos in women undergoing *in vitro* fertilization. *Fertil Steril* 2007;**88**:1350–1357.

Seli E, Vergouw CG, Morita H, Botros L, Roos P, Lambalk CB, Yamashita N, Kato O, Sakkas D. Noninvasive metabolomic profiling as an adjunct to morphology for noninvasive embryo assessment in women undergoing single embryo transfer. *Fertil Steril* 2010;**94**:535–542.

Seli E, Bruce C, Botros L, Henson M, Roos P, Judge K, Hardarson T, Ahlstrom A, Harrison P, Henman M *et al.* Receiver operating characteristic (ROC) analysis of day 5 morphology grading and metabolomic viability score on predicting implantation outcome. *J Assist Reprod Genet* 2011;**28**:137–144.

Shea JL, Diamandis EP, Hoffman B, Lo YM, Canick J, van den Boom D. A new era in prenatal diagnosis: the use of cell-free fetal DNA in maternal circulation for detection of chromosomal aneuploidies. *Clin Chem* 2013;**59**:1151–1159.

Si H, Sun X, Chen Y, Cao Y, Chen S, Wang H, Hu C. Circulating microRNA-92a and microRNA-21 as novel minimally invasive biomarkers for primary breast cancer. *J Cancer Res Clin Oncol* 2013;**139**:223–229.

Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet* 2005;**365**:785–799.

Silvestri P, Di Russo C, Rigattieri S, Fedele S, Todaro D, Ferraiuolo G, Altamura G, Loschiavo P. MicroRNAs and ischemic heart disease: towards a better comprehension of pathogenesis, new diagnostic tools and new therapeutic targets. *Recent Pat Cardiovasc Drug Discov* 2009;**4**:109–118.

Singh PK, Singh AV, Chauhan DS. Current understanding on micro RNAs and its regulation in response to Mycobacterial infections. *J Biomed Sci* 2013;**20**:14.

Sirichotiyakul S, Charoenkwan P, Sanguansermsri T. Prenatal diagnosis of homozygous alpha-thalassemia-1 by cell-free fetal DNA in maternal plasma. *Prenat Diagn* 2012; **32**:45–49.

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

Sirotkin AV, Laukova M, Ovcharenko D, Brenaut P, Mlyncek M. Identification of microRNAs controlling human ovarian cell proliferation and apoptosis. *J Cell Physiol* 2010;**223**:49–56.

Sohel MM, Hoelker M, Noferesti SS, Salilew-Wondim D, Tholen E, Looft C, Rings F, Uddin MJ, Spencer TE, Schellander K *et al*. Exosomal and non-exosomal transport of extra-cellular microRNAs in follicular fluid: implications for bovine oocyte developmental competence. *PLoS ONE* 2013;**8**:e78505.

Sparks AB, Struble CA, Wang ET, Song K, Oliphant A. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012;**206**:319 e1–9.

Spencer K, de Kok JB, Swinkels DW. Increased total cell-free DNA in the serum of pregnant women carrying a fetus affected by trisomy 21. *Prenat Diagn* 2003; **23**:580–583.

Stigliani S, Anserini P, Venturini PL, Scaruffi P. Mitochondrial DNA content in embryo culture medium is significantly associated with human embryo fragmentation. *Hum Reprod* 2013;**28**:2652–2660.

Sugiura K, Pendola FL, Eppig JJ. Oocyte control of metabolic cooperativity between oocytes and companion granulosa cells: energy metabolism. *Dev Biol* 2005; **279**:20–30.

Suh N, baenher L, Moltzahn F, Melton C, Shenoy A, Chen J, Blelloch R. MicroRNA function is globally suppressed in mouse oocytes and early embryos. *Curr Biol* 2010;**20**:271–277.

Sun M, Gomes S, Chen P, Frankenberger CA, Sankarasharma D, Chung CH, Chada KK, Rosner MR. RKIP and HMGA2 regulate breast tumor survival and metastasis through lysyl oxidase and syndecan-2. *Oncogene* 2013. doi:10.1038/onc.2013.328.

Swinkels DW, de Kok JB, Hendriks JC, Wiegerinck E, Zusterzeel PL, Steegers EA. Hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome as a complication of preeclampsia in pregnant women increases the amount of cell-free fetal and maternal DNA in maternal plasma and serum. *Clin Chem* 2002; **48**:650–653.

Takanabe R, Ono K, Abe Y, Takaya T, Horie T, Wada H, Kita T, Satoh N, Shimatsu A, Hasegawa K. Up-regulated expression of microRNA-143 in association with obesity in adipose tissue of mice fed high-fat diet. *Biochem Biophys Res Commun* 2008; **376**:728–732.

Tanaka H, Tsuda H, Nishimura S, Nomura H, Kataoka F, Chiyoda T, Tanaka K, Iguchi Y, Susumu N, Aoki D. Role of circulating free alu DNA in endometrial cancer. *Int J Gynecol Cancer* 2012;**22**:82–86.

Tang F, Kaneda M, O'Carroll D, Hajkova P, Barton SC, Sun YA, Lee C, Tarakhovsky A, Lao K, Surani MA. Maternal microRNAs are essential for mouse zygotic development. *Genes Dev* 2007;**21**:644–648.

Thomas M, Lieberman J, Lal A. Desperately seeking microRNA targets. *Nat Struct Mol Biol* 2010;**17**:1169–1174.

Thery C. Exosomes: secreted vesicles and intercellular communications. *F1000 Biol Rep* 2011;**3**:15.

Tijsen AJ, Creemers EE, Moerland PD, de Windt LJ, van der Wal AC, Kok WE, Pinto YM. MiR423-5p as a circulating biomarker for heart failure. *Circ Res* 2010; **106**:1035–1039.

Trajkovski M, Hausser J, Soutschek J, Bhat B, Akin A, Zavolan M, Heim MH Stoffel M. MicroRNAs 103 and 107 regulate insulin sensitivity. *Nature* 2011; **474**:649–653.

Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. *Nucleic Acids Res* 2011;**39**:7223–7233.

Tzur G, Levy A, Meiri E, Barad O, Spector Y, Bentwich Z, Mizrahi L, Katzenellenbogen M, Ben-Shushan E, Reubinoff BE *et al*. MicroRNA expression patterns and function in endodermal differentiation of human embryonic stem cells. *PLoS ONE* 2008;**3**:e3726.

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

van Rooij E, Olson EN. MicroRNAs put their signatures on the heart. *Physiol Genomics* 2007;**31**:365–366.

van Schooneveld E, Wouters MC, Van der Auwera I, Peeters DJ, Wildiers H, Van Dam PA, Vergote I, Vermeulen PB, Dirix LY, Van Laere SJ. Expression profiling of cancerous and normal breast tissues identifies microRNAs that are differentially expressed in serum from patients with (metastatic) breast cancer and healthy volunteers. *Breast Cancer Res* 2012;**14**:R34.

Verghese ET, Hanby AM, Speirs V, Hughes TA. Small is beautiful: microRNAs and breast cancer—where are we now? *J Pathol* 2008;**215**:214–221.

Verweij EJ, Jacobsson B, van Scheltema PA, de Boer MA, Hoffer MJ, Hollemon D, Westgren M, Song K, Oepkes D. European non-invasive trisomy evaluation (EU-NITE) study: a multicenter prospective cohort study for non-invasive fetal trisomy 21 testing. *Prenat Diagn* 2013;**33**:996–1001.

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

Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Mullerian hormone: a new marker for ovarian function. *Reproduction* 2006;**131**:1–9.

Vora NL, Johnson KL, Basu S, Catalano PM, Hauguel-De Mouzon S, Bianchi DW. A multifactorial relationship exists between total circulating cell-free DNA levels and maternal BMI. *Prenat Diagn* 2012;**32**:912–914.

Walsh JM, Goldberg JD. Fetal aneuploidy detection by maternal plasma DNA sequencing: a technology assessment. *Prenat Diagn* 2013;**33**:514–520.

Wang F, Zheng Z, Guo J, Ding X. Correlation and quantitation of microRNA aberrant expression in tissues and sera from patients with breast tumor. *Gynecol Oncol* 2010a; **119**:586–593.

Wang GK, Zhu JQ, Zhang JT, Li Q, Li Y, He J, Qin YW, Jing Q. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J* 2010b;**31**:659–666.

Wang JF, Yu ML, Yu G, Bian JJ, Deng XM, Wan XJ, Zhu KM. Serum miR-146a and miR-223 as potential new biomarkers for sepsis. *Biochem Biophys Res Commun* 2010c;**394**:184–188.

Wang K, Zhang S, Weber J, Baxter D, Galas DJ. Export of microRNAs and microRNA-protective protein by mammalian cells. *Nucleic Acids Res* 2010d; **38**:7248–7259.

Wang SJ, Gao ZY, Lu YP, Li YL, You YQ, Zhang LW, Wang LX, Xu H. Value of detection of cell-free fetal DNA in maternal plasma in the prenatal diagnosis of chromosomal abnormalities. *Zhonghua Fu Chan Ke Za Zhi* 2012a;**47**:808–812.

Wang X, Wu X, Yan L, Shao J. Serum miR-103 as a potential diagnostic biomarker for breast cancer. *Nan Fang Yi Ke Da Xue Xue Bao* 2012b;**32**:631–634.

Wang XW, Heegaard NH, Orum H. MicroRNAs in liver disease. *Gastroenterology* 2012c;**142**:1431–1443.

Wang PY, Gong HT, Li BF, Lv CL, Wang HT, Zhou HH, Li XX, Xie SY, Jiang BF. Higher expression of circulating miR-182 as a novel biomarker for breast cancer. *Oncol Lett* 2013a;**6**:1681–1686.

Wang WT, Zhao YN, Han BW, Hong SJ, Chen YQ. Circulating microRNAs identified in a genome-wide serum microRNA expression analysis as noninvasive biomarkers for endometriosis. *J Clin Endocrinol Metab* 2013b;**98**:281–289.

Wataganara T, Bianchi DW. Fetal cell-free nucleic acids in the maternal circulation: new clinical applications. *Ann N Y Acad Sci* 2004;**1022**:90–99.

Wataganara T, LeShane ES, Farina A, Messerlian GM, Lee T, Canick JA, Bianchi DW. Maternal serum cell-free fetal DNA levels are increased in cases of trisomy 13 but not trisomy 18. *Hum Genet* 2003;**112**:204–208.

Webb RL, Murphy MP. β-secretases, Alzheimer's disease, and Down syndrome. *Curr Gerontol Geriatr Res* 2012;**2012**:362839.

Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ, Wang K. The microRNA spectrum in 12 body fluids. *Clin Chem* 2010;**56**:1733–1741.

Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BC, Themmen AP. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod* 2004;**10**:77–83.

Weiler J, Hunziker J, Hall J. Anti-miRNA oligonucleotides (AMOs): ammunition to target miRNAs implicated in human disease? *Gene Ther* 2006;**13**:496–502.

Wilfred BR, Wang WX, Nelson PT. Energizing miRNA research: a review of the role of miRNAs in lipid metabolism, with a prediction that miR-103/107 regulates human metabolic pathways. *Mol Genet Metab* 2007;**91**:209–217.

Wong SS, Ritner C, Ramachandran S, Aurigui J, Pitt C, Chandra P, Ling VB, Yabut O, Bernstein HS. miR-125b promotes early germ layer specification through Lin28/let-7d and preferential differentiation of mesoderm in human embryonic stem cells. *PLoS ONE* 2012;**7**:e36121.

Wright CF, Burton H. The use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis. *Hum Reprod Update* 2009;**15**:139–151.

Wright CF, Wei Y, Higgins JP, Sagoo GS. Non-invasive prenatal diagnostic test accuracy for fetal sex using cell-free DNA a review and meta-analysis. *BMC Res Notes* 2012;**5**:476.

Wu Q, Lu Z, Li H, Lu J, Guo L, Ge Q. Next-generation sequencing of microRNAs for breast cancer detection. *J Biomed Biotechnol* 2011;**2011**:597145.

Wu L, Zhou H, Lin H, Qi J, Zhu C, Gao Z, Wang H. Circulating microRNAs are elevated in plasma from severe preeclamptic pregnancies. *Reproduction* 2012;**143**:389–397.

Wu C, Ding X, Li H, Zhu C, Xiong C. Genome-wide promoter methylation profile of human testis and epididymis: identified from cell-free seminal DNA. *BMC Genomics* 2013a;**14**:288.

Wu H, Xiao Z, Wang K, Liu W, Hao Q. MiR-145 is downregulated in human ovarian cancer and modulates cell growth and invasion by targeting p70S6K1 and MUC1. *Biochem Biophys Res Commun* 2013b;**441**:693–700.

Xie H, Lim B, Lodish HF. MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity. *Diabetes* 2009;**58**:1050–1057.

Xu S, Linher-Melville K, Yang BB, Wu D, Li J. Micro-RNA378 (miR-378) regulates ovarian estradiol production by targeting aromatase. *Endocrinology* 2011a;**152**:3941–3951.

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

Yan G, Zhang L, Fang T, Zhang Q, Wu S, Jiang Y, Sun H, Hu Y. MicroRNA-145 suppresses mouse granulosa cell proliferation by targeting activin receptor IB. *FEBS Lett* 2012;**586**:3263–3270.

Yang Y, Bai W, Zhang L, Yin G, Wang X, Wang J, Zhao H, Han Y, Yao YQ. Determination of microRNAs in mouse preimplantation embryos by microarray. *Dev Dyn* 2008;**237**:2315–2327.

Yang X, Zhou Y, Peng S, Wu L, Lin HY, Wang S, Wang H. Differentially expressed plasma microRNAs in premature ovarian failure patients and the potential regulatory function of mir-23a in granulosa cell apoptosis. *Reproduction* 2012;**144**:235–244.

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**:126.

Yao N, Lu CL, Zhao JJ, Xia HF, Sun DG, Shi XQ, Wang C, Li D, Cui Y, Ma X. A network of miRNAs expressed in the ovary are regulated by FSH. *Front Biosci (Landmark Ed)* 2009;**14**:3239–3245.

Yao G, Yin M, Lian J, Tian H, Liu L, Li X, Sun F. MicroRNA-224 is involved in transforming growth factor-beta-mediated mouse granulosa cell proliferation and granulosa cell function by targeting Smad4. *Mol Endocrinol* 2010a;**24**:540–551.

Yao N, Yang BQ, Liu Y, Tan XY, Lu CL, Yuan XH, Ma X. Follicle-stimulating hormone regulation of microRNA expression on progesterone production in cultured rat granulosa cells. *Endocrine* 2010b;**38**:158–166.

Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 2003;**17**:3011–3016.

Yin M, Lu 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.

Zachariah RR, Schmid S, Buerki N, Radpour R, Holzgreve W, Zhong X. Levels of circulating cell-free nuclear and mitochondrial DNA in benign and malignant ovarian tumors. *Obstet Gynecol* 2008;**112**:843–850.

Zen K, Zhang CY. Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. *Med Res Rev* 2012;**32**:326–348.

Zeng RC, Zhang W, Yan XQ, Ye ZQ, Chen ED, Huang DP, Zhang XH, Huang GL. Down-regulation of miRNA-30a in human plasma is a novel marker for breast cancer. *Med Oncol* 2013;**30**:477.

Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, Hristov M, Köppel T, Jahantigh MN, Lutgens E *et al.* Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci Signal* 2009;**8**:2.

Zhang Y, Liu D, Chen X, Li J, Li L, Bian Z, Sun F, Lu J, Yin Y, Cai X *et al.* Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Mol Cell* 2010;**39**:133–144.

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 (Landmark Ed)* 2013a;**18**:588–597.

Zhang Q, Hu G, Yang Q, Dong R, Xie X, Ma D, Shen K, Kong B. A multiplex methylation-specific PCR assay for the detection of early-stage ovarian cancer using cell-free serum DNA. *Gynecol Oncol* 2013b;**130**:132–139.

Zhang Q, Liu H, McGee J, Walsh EJ, Soukup GA, He DZ. Identifying microRNAs involved in degeneration of the organ of corti during age-related hearing loss. *PLoS ONE* 2013c;**8**:e62786.

Zhao H, Rajkovic A. MicroRNAs and mammalian ovarian development. *Semin Reprod Med* 2008;**26**:461–468.

Zhao Z, Zhao Q, Warrick J, Lockwood CM, Woodworth A, Moley KH, Gronowski AM. Circulating microRNA miR-323-3p as a biomarker of ectopic pregnancy. *Clin Chem* 2012;**58**:896–905.

Zheng H, Zhang L, Zhao Y, Yang D, Song F, Wen Y, Hao Q, Hu Z, Zhang W, Chen K. Plasma miRNAs as diagnostic and prognostic biomarkers for ovarian cancer. *PLoS ONE* 2013;**8**:e77853.

Zhong XY, Burk MR, Troeger C, Kang A, Holzgreve W, Hahn S. Fluctuation of maternal and fetal free extracellular circulatory DNA in maternal plasma. *Obstet Gynecol* 2000;**96**:991–996.

Zhong XY, Laivuori H, Livingston JC, Ylikorkala O, Sibai BM, Holzgreve W, Hahn S. Elevation of both maternal and fetal extracellular circulating deoxyribonucleic acid concentrations in the plasma of pregnant women with preeclampsia. *Am J Obstet Gynecol* 2001;**184**:414–419.

Zhong XY, Holzgreve W, Hahn S. The levels of circulatory cell free fetal DNA in maternal plasma are elevated prior to the onset of preeclampsia. *Hypertens Pregnancy* 2002;**21**:77–83.

Zimmermann BG, Maddocks DG, Avent ND. Quantification of circulatory fetal DNA in the plasma of pregnant women. *Methods Mol Biol* 2008;**444**:219–229.

Zimmermann B, Hill M, Gemelos G, Demko Z, Banjevic M, Baner J, Ryan A, Sigurjonsson S, Chopra N, Dodd M *et al.* Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. *Prenat Diagn* 2012;**32**:1233–1241.

Zubakov D, Boersma AW, Choi Y, van Kuijk PF, Wiemer EA, Kayser M. MicroRNA markers for forensic body fluid identification obtained from microarray screening and quantitative RT-PCR confirmation. *Int J Legal Med* 2010;**124**:217–226.