miRNAs as a novel clinical biomarker and therapeutic targets in polycystic ovary syndrome (PCOS): a review

Running title: miRNAs in PCOS

Authors:

Dr Mohammed Abdalla

Hull York Medical School, Academic Diabetes, Endocrinology and Metabolism, University of Hull, Hull, UK. Email: M.a.abdalla-2019@hull.ac.uk.

Dr Harshal Deshmukh

Clinical lecturer at Hull York Medical School, Academic Diabetes, Endocrinology and Metabolism, University of Hull, Hull, UK. Email: Harshaldeshmukh@nhs.net.

Professor Stephen L Atkin

Head of School postgraduate studies and research, RCIS-Bahrain, Medical University of Bahrain, Kingdom of Bahrain. Email: satkin@rcis-mub.com.

Professor Thozhukat Sathyapalan (Correspondent author)

Honorary consultant Endocrinologist at Hull University Teaching Hospital NHS Trust, UK. Chair in Academic Diabetes, Endocrinology and metabolism in Hull York Medical School, University of Hull, UK. Tel: 01482675340. Email: Thozhukat.Sathyapalan@hyms.ac.uk.

Abstract

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder in females of the reproductive age. PCOS is commonly manifested as ovulatory dysfunction, clinical and biochemical excess androgen level, and polycystic ovaries. Metabolic sequelae associated with PCOS, including insulin resistance (IR), type 2 diabetes (T2DM), obesity and increased cardiometabolic risk. The underlying pathology of PCOS is not fully understood with various genetic and environmental factors have been proposed. MicroRNAs (miRNAs), are endogenously produced, small non-coding, single-stranded RNAs that capable of regulating gene expression at the post-transcriptional level. Altered miRNAs expression has been associated with various disorders, including T2DM, IR, lipid disorder, infertility, atherosclerosis, endometriosis, and cancer.

Given that PCOS also present with similar features, there is an increasing interest to investigate the role of miRNAs in the diagnosis and management of PCOS. In recent years, studies have demonstrated that miRNAs are present in various body fluids, including follicular fluid of women with PCOS. Therefore, it may act as a potential biomarker and could serve as a novel therapeutic target for the diagnosis and treatment of PCOS. This review aims to summarise the up to date research on the relation between miRNAs and PCOS and explore its potential role in the diagnosis and the management of PCOS.

Keywords: polycystic ovary syndrome, PCOS, microRNA, miRNAs, biomarkers, T2DM, infertility, follicular development, lipid metabolism, hyperandrogenism.

Total words count 8094 words.

Conflict of interest: The authors have no conflicts of interest to declare**.**

Statement of Funding: The authors have not received any kind of fund related to the review.

Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder in women of reproductive age with a prevalence of approximately 10 - 20 % (1). It is a heterogeneous disorder with different phenotypes and characterised by clinical and biochemical evidence of hyperandrogenism, menstrual irregularities and polycystic ovarian morphology (2). The Rotterdam 2003 criteria are currently acceptable for the diagnosis of PCOS, where two out of the following three criteria are satisfied: anovulation/oligoovulation, clinical and biochemical sign of hyperandrogenism and polycystic ovaries, after excluding other aetiologies such as congenital adrenal hyperplasia and androgen-secreting tumours (3). PCOS also has its metabolic consequences such as insulin resistance (IR), hyperlipidaemia, obesity, oxidative stress, type 2 diabetes mellitus (T2DM) and increased risk of cardiovascular disease (CVD) (4). It also linked to an increase in pregnancy-related complications such as gestational diabetes, preterm birth, antepartum haemorrhage and pregnancy-induced hypertension (5). The aetiology of PCOS is unclear; however, environmental and genetic factors have been proposed as a potential cause of PCOS (6). In recent years, the association between regulatory miRNAs and various diseases has been an area of intensive research.

MicroRNAs (miRNAs) are a new class of endogenous, non-coding, single-stranded RNA molecules with 20-25 nucleotides that regulate post-transcriptional gene expression by binding to the 3' untranslated location of the target messenger RNA (mRNA), thus, lead to the inhibition of mRNA expression and block post-transcriptional protein translation (7, 8). miRNAs are widely presented in the human body and can be isolated from urine, plasma, semen and saliva or might be encapsulated in microvesicles (9-12). They have also been expressed in different organs, including the liver, adipose tissue and muscle (13)**—figure 1**. A piece of accumulative evidence has shown that miRNAs regulate various critical regulatory biological functions including cell growth and development, apoptosis, metabolism, stress response and hematopoietic differentiation (8, 14). A single miRNA has the potential to modulate the function and expression of various target genes, and amplification or inhibition of miRNA signal via the regulatory feedback mechanism may drive to a significant alteration of miRNA expression which contributes to different disease including ovarian cancer, endometriosis, cardiovascular disease and inadequate ovarian response (15-17). There is also growing evidence demonstrating the influence of miRNAs in the pathogenesis of diabetes mellitus, and they could potentially be a novel biomarker for diabetes (18). There is also data showing differential expression of circulating miRNAs in women with and without PCOS (19). Therefore, it has been proposed to be useful as a diagnostic biomarker or a potential therapeutic target for PCOS. However, our understanding of the exact relationship between miRNAs and PCOS is still preliminary, and the potential role of miRNAs in the diagnosis and the management of PCOS is yet to be clarified.

In line with these considerations, this review was conducted to summarise the existing data to establish the potential role of some miRNAs as a potential clinical biomarker and therapeutic targets for the diagnosis and the management of PCOS (**Table 1**).

miRNAs as a potential novel clinical biomarker for PCOS

miRNAs and ovarian dysfunction in PCOS

In recent years, many attempts have been made trying to understand the exact mechanism of anovulation and abnormal folliculogenesis in women with PCOS. As a result, Increased plasm level of luteinising hormone (LH) and androgen with normal or low levels of the follicular stimulating hormone (FSH) has been suggested (20). Furthermore, abnormal steroidogenesis, excessive expression of anti-Mullerian hormone (AMH) and impaired follicular apoptosis have also been reported (21).

MiRNAs increase expression of proliferating cell nuclear antigen protein (PCNA), a marker of proliferation (22). They also regulate follicular granulosa cells (FGCs) by modulating expression at the target organ (23), and they are differentially expressed amongst different follicular sizes during follicular atresia (24, 25). Among the most common miRNAs altered during follicular atresia is miR-1275, which is also known to regulate FGCs apoptosis (26). In several human studies, a vast array of miRNAs has been explored to determine their

functions in follicular atresia and FGCs apoptosis. The miR-15a has been found promotes steroidogenesis by increasing progesterone and testosterone synthesis (27). **Figure 1**. The miR-23a and miR-27a stimulate FGCs apoptosis by targeting SMAD5 protein, while miR-93 promotes proliferation by targeting cyclin-dependent kinase inhibitor 1A (CDKN1A) protein (28-30). The Let-7 family of miRNAs which regulates cell proliferation, differentiation and tumour suppression was highly expressed among animal species (31). Further study has reported the transforming growth factor β receptor (TGFBR), and the mitogenic-activated protein kinase 1 (MAP3K1) as a potential target for miR-let-7 and the suppression of MAP3K1 induces apoptosis (32). The miR-Let-7c, miR-23A, miR-27a and miR-22-3p were also expressed in patients with premature ovarian failure compared to the healthy population (33). Most recent studies have shown abnormal expression of miRNAs are often seen with follicular maturation in PCOS (14, 34, 35).

In a study of rat model with PCOS exposed to dihydrotestosterone (DHT) found that 72 miRNA was upregulated and the 17 miRNA were downregulated in DHT- exposed ovaries compared to control ovaries, with miR-32, miR-21, miR-182, miR-183, miR-184 and miR-96 were primarily downregulated (36). Furthermore, most of these miRNAs were extensively expressed in granulosa cells (GCs) of the ovary compared to other ovarian cells. For example, miRNA-376 associated with primordial follicular development and it influences GCs proliferation through miRNA-376a, which directly binds to the targeted location 3'UTR mRNA of the PCNA (37). **Table1**. However, further study showed that increased expression of miRNA-143 inhibits primordial folliculogenesis by suppressing GCs proliferation (38). miR-224 also has been expressed in GCs of the ovaries; it induces GCs proliferation via

transforming growth factor-β (TGF-β), and induction of GCs proliferation mediated through TGF-β 1 receptor facilitated by miR-224 upregulation (39).

Furthermore, miR-145 found to target the transforming growth factor β 2 (TGF-β 2) receptor and hence initiates and maintains the primordial follicular development (34). miR-224 has also been recognised targeting Pentraxin 3 (PTX3), a protein linked to cumulus expansion (40). In PCOS, a miRNA-PTX3 expression associated with the fertilisation process and therefore; it could potentially be used as a biomarker to assess the quality of oocyte (41). miR-182 and miR-15a play an essential role in the physiology of GCs of the ovaries by regulating steroidogenesis, induce proliferation and apoptosis; however, their levels were significantly low in the ovarian cell of PCOS rat model (27, 36).**Figure 1**. Thus, the expression of these miRNAs might influence the timing of development and maturation of oocyte by targeting the gonadotropin-releasing hormone (GnRH) pathway (42). Therefore, these findings outlined the importance of miRNAs for controlling the process of proliferation and apoptosis of the ovarian GCs and subsequently folliculogenesis, which could be a potential target to assess for ovulation in PCOS.

miRNAs and follicular fluid (FF) in PCOS

Follicular fluid (FF) provides a suitable environment for oocyte development and maturation. Its proximity to the oocytes allows for efficient exchange of components between blood, granulosa and theca cells (TCs) (43). Additionally, FF contains various hormones such as androgen, oestrogen, LH, FSH, growth hormone, TGF-β, anti-Mullerian hormone (AMH), activin and metabolic and secretory products of the oocyte (44). The collection of FF is relatively easy during harvesting oocyte for assisted fertilisation, i.e.

in-vitro fertilisation (IVF). Thus, it serves as a less invasive procedure for obtaining miRNAs and to assessment for fertility outcomes.

Furthermore, analysing FF compositions may also indicate the quality of oocyte and the functional status of GCs and CTs (45). The recent discovery of miRNAs in the human body has inspired researchers to study their functions in various biological processes. In a study by Butler et al., has detected 176 miRNAs, of which 29 were differentially expressed in the FF of women with PCOS and normal control women (46). As a result, miR-382-5p was correlated positively with age and free androgen index (FAI), miR-199b-5p linked with AMH and miR-127-3p was associated with insulin resistance, and further analysis revealed 12 miRNAs correlated with reproductive pathways (46). In the previous study by Sathyapalan et al. has described the potential diagnostic significance of miR-93 as a novel biomarker for the diagnosis of PCOS after it was found relatively higher in women with PCOS compared to women without PCOS (47). A further study discovered over 100 differentially expressed miRNAs that potentially regulate steroidogenesis in FF of women with PCOS with two miRNAs (miR-132 and miR-320) has significantly reduced in FF of PCOS women (43).

Moreover, downregulation of miR-29a, miR-24-3p and miR-574-3p were reported in women with PCOS compared with women without PCOS, and serum levels of total and free androgen were correlated positively with miR-518f-3p in subjects with PCOS (48). These alterations in miRNAs profiles might facilitate the phenotypic stratification in women with PCOS. The combination of miR-30a, Let-7b and miR-140 expression has a sensitivity of 70 % and specificity of over 83 % in discriminating between the normal ovarian reserve and PCOS, particularly during assisted fertilisation (49). Thus, this could potentially provide a novel biomarker to predict outcomes and to facilitate a personalised level of medical care for

women with PCOS. In a study in which 27 miRNAs were differentially expressed in women with PCOS, miR-92a and miR-92b were significantly downregulated (50). Another study reported expression of 235 miRNAs of which 29 miRNAs were differentially expressed in PCOS and control group, but miR-32, miR-34c, miR-135a, miR-18b and miR-9 have demonstrated a significantly increased expression in PCOS group (51). To sum, these findings confirm that there are differentially expressed miRNAs in the FF of women with PCOS. Thus, it is imperative to propose that examining different miRNAs present in the FF of women with PCOS may potentially provide a novel biomarker for the diagnosis of PCOS. Furthermore, it might aid with the classification of different phenotypes of PCOS. Moreover, the environment of FF also contains various hormones and metabolic products; adjusting this microenvironment could improve the reproductive outcomes in PCOS.

miRNAs and fertility in PCOS

The role of miRNAs in the level of fertility has recently been studied extensively, particularly after the discovery of Dicer 1 a ribonuclease III enzyme essential for miRNAs production (52). The Knock-out of this enzyme in mice resulted in infertility by reducing the rate of ovulation and mitogenic progression due to defective spindle arrangement in an animal model (53). However, in a human study, blastocytes extraction from women with PCOS has shown significantly low expression of hsa-miR-19a, hsa-miR-19b, hsa-miR-24 and hsa-miR-93 compared to healthy control. **Figure 1**. Furthermore, heatmap analysis for the expression of these miRNAs showed expression of miR-19a and its target gene ARIH2 (essential for cell differentiation) has significantly upregulated in women with PCOS compared to women without PCOS.

Similarly, NFAT5 and KHSRP genes targeted by miR-24 and encoding transcriptional and decaying factors for miRNAs were both upregulated in women with PCOS with a significant decrease in miR-24 expression (54). miR-290-295 have a pivotal role in the embryogenesis as demonstrated in a cluster of mutant mouse embryos, in the miR-290-295 deficient male mutant mice the fertility was restored later during their development, unlike the female mutant mice which remained infertile, indicating the role of defective miR-290-295 on the GCs (55). Moreover, transfection of GCs with miR-27a, miR-322 and Let-7c inhibitor has led to increased oocyte follicular maturation of mouse ovaries (56).

Maternal age is another major factor for infertility. In a study, various differentially expressed miRNAs were expressed in women above the age of 40s compared to women in their 20s and miR-93 was exceptionally expressed only in blastocytes with chromosomal abnormalities in older women (57), this is a clear indication that maternal ageing is not only a risk factor for infertility but also associated with modification of miRNAs profiles (58).

miRNAs and steroidogenesis in PCOS

The reproductive cycle of the ovaries is coordinated by hormones released from the hypothalamic-pituitary-ovarian axis. Alterations in this pathway lead to abnormal hormone production, which has been observed in women with PCOS (59). Excess androgen level is a common presenting feature in PCOS, and it may be due to overstimulation of the ovarian TCs by LH to synthesise androgen or due to defects of androgen receptors at the target organs level (60). The majority of testosterone is bound to SHBG and albumin with the only small fraction is circulating freely as bioactive testosterone. Women with PCOS have low SHBG levels which increase the bioavailable testosterone level (61). There is a considerable amount of evidence proposed that excess androgen is the main drive for ovulatory and

metabolic dysfunction seen in PCOS (62). It facilitates visceral adiposity and IR with subsequent increase in ovarian androgen production and commonly present as hirsutism, acne and menstrual disturbances (63). Even though high androgen is a pathological feature of PCOS, recent evidence suggested that testosterone, dihydrotestosterone (DHT) and androstenedione (A4) play a vital role on ovulation by facilitating the follicular development and maturation (64). Androgen hormones apply their function by binding into the androgen receptor (AR) at their target tissues, and increased expression of AR has been shown in women with PCOS (65). AR can be expressed in various cells; however, it is predominantly found in the granulosa cells (GCs) of the growing ovarian follicle (66). The effects of miRNAs on steroidogenesis from the ovarian cells have been explored across a variety of the living animal species.

The transfection of miR-24 resulted in a decreased level of oestradiol secretion. Conversely, overexpression of miR-520c-3p, miR-132 and miR-320 derived to increased oestradiol release and the transfection of miR-483-5p, miR-24 and miR-193b associated with decreased progesterone secretion (43). miR-513a-3p was negatively correlated with the luteinising hormone and gonadotropin receptor (LHCGR) (67). Furthermore, miR-107 positively associated with testosterone secretion; on the other hand, miR-146a has significantly reduced testosterone secretion (19, 68). miR-103, miR-155 and miR-21 were also shown to correlate positively with free testosterone levels in women with PCOS (69). miR-320, miR-518 and miR-29a were positively associated with an increased level of serum testosterone, **Figure 1**. while miR-151 was negatively linked to serum testosterone (70). Recently, a study suggested that miR-155 and miR-29a are negatively associated with serum A4 in women with PCOS (71).

The synthesis of steroid hormones depends on different genes which regulate the signalling pathways, androgen metabolism and lipid transport. At the same time, LH and FSH control the androgen production and the testosterone conversion respectively by acting directly on the GCs of the ovarian follicles. Key enzymes and genes such as CYP19, CYP11A, StAR, CYP17 CYP19A1 and 3-b HSD are involved in the steroid hormone production, and oestrogen synthesis depends on aromatase enzyme which regulated by CYP19 A1 gene (60).

Overexpression of miR-181a and miR-378 downregulate aromatase enzyme and hence reduce oestrogen synthesis in GCs (72-74). Inversely, many miRNAs have shown to correlate positively with oestradiol synthesis. For instance, overexpression of miR-133b increase oestradiol synthesis with a simultaneous increase in CYP19A1 in the GCs of FSH-stimulated mice by targeting forkhead box L2 (fox12) (75, 76).**Figure1.** On the other hand, overexpression of miR-224 derived to increased oestrogen release by targeting SMAD4 of mouse GCs (39). miR-193a-5p and miR-199a-3p are negatively correlated with testosterone level and positively linked with SHBG and oestradiol in women with PCOS (69). In-depth understanding of the correlation between miRNAs and the synthesis of steroid hormones will potentially aid the diagnosis of PCOS and will help predict its metabolic consequences.

miRNAs and the metabolic consequences of the PCOS

miRNAs and insulin resistance in PCOS

Insulin resistance (IR) is a common feature of PCOS with a prevalence of approximately 70 % of the cases have IR (77). It plays a significant role in the pathogenesis of PCOS and associated with increased risk of metabolic syndrome, impaired glucose tolerance, dyslipidaemia, T2DM and cardiovascular disorders (78). Hyperinsulinemia is capable of

stimulating steroidogenesis and increase ovarian androgen secretion from the theca cells (TCs) mediated by the insulin growth factor-1 (IGF-1) receptors; high insulin level also potentiates LH effect on TCs to cause excess androgen (77). **Figure2.** IR is contributing to high androgen level by increased activity of CYP17 enzyme with synergetic action of LH on TCs. It increases cyclic adenosine monophosphate (cAMP) concentration, reduces SHBG and subsequent increase the free testosterone levels (79, 80). In an animal study by Ling et al., 3T3-L1 adipocyte cells were transformed into IR cells by administering high levels of insulin and glucose after a significant increase in expression of miR-320 (81). However, insulin sensitivity was restored shortly after treatment with anti-miR-320 oligos which alleviated IR by upregulating glucose transporter 4 (GLUT4) expression and improving insulin-mediated glucose uptake (82). miR-320 has been confirmed to exist in abundance in FF of women with PCOS, advocating that it could be a target for enhancing insulin sensitivity (43, 81). A study found that expression of miR-194,miR-193b and miR-122 was upregulated in women with PCOS particularly those with impaired glucose by targeting different signalling pathways, including insulin signalling pathway, glycometabolism pathway and follicular development pathway (83). The role of miRNAs in regulating GLUT4 has been recently investigated. Expression of miR-93 showed a strong correlation between GLUT4 and IR in adipose tissue of women with PCOS, and activating miR-93 downregulates GLUT4 by targeting GLUT4 3'UTR; however, suppressing miR-93 activity facilitated GLUT4 expression (84). Overexpression of miR-33b-5p was detected in the ovarian cells of PCOS rat model with IR, and it was negatively correlated with GLUT4, sterol regulatory element-binding protein 1 (SREBF1) and high mobility group A2 (HMGA2) expression. These findings demonstrated that miR-33b-5p plays a vital role in the development of IR in patients with PCOS through inhibition of GLUT4 expression. Additionally, it also has the potential to adjust

the expression of various protein cascades in insulin signalling pathways (85). For example, the expression of miR-143 inhibits insulin-regulated AKT-kinase activity a key enzyme in the insulin signalling pathway (86). Many miRNAs such as miR-126, miR-29, miR-1 and miR-19a have been proposed to regulate PI3K, which mediate insulin-facilitated glucose uptake (86).**Figure2**.

Furthermore, the miR-483-5p reduces IR and facilitates cumulous cell proliferation by activating PI3K/AKT (87). In general, the expression of miRNAs plays an essential role in regulating glucose metabolism, insulin signalling pathway and the pathogenesis of IR in women with PCOS by determining the expression of GLUT4, proteins and enzymes of the glucose metabolism.

miRNAs and lipid disorders in PCOS

PCOS also associated with dyslipidaemia with around 70 % of women with PCOS have some sort of abnormal lipid profiles manifesting as high triglycerides, elevated low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) levels (88, 89). These high lipid profiles are atherogenic and strongly associated with a high risk of CVD; therefore, women with PCOS are at increased risk of cardiovascular morbidities (90). Obesity also has a detrimental effect on the metabolic aspects of PCOS with approximately around 88 % of women with PCOS, either overweight or obese (89).

It is well established that miRNAs have significant effects on lipid metabolism and cholesterol homeostasis. The miR-33 has been shown to target adenosine triphosphate (ATP) binding cascade transporter A1 (ABCA1) an important regulator which increases the level of HDL- C and facilitates cholesterol disposal by the liver (91-93). Moreover, miR-33

has also shown to regulates ABCG1, cholesterol 7-α hydroxylase (CYP7A1) and ABCB11 genes involved in reverse cholesterol transport (RCT) (94, 95). **Figure 1.** The miR-122 and miR-30c play an essential role in controlling LDL-C by adjusting the cholesterol biosynthesis and VLDL-C secretion. Even more, they decrease Apo B lipoproteins by targeting the microsomal triglyceride transferase protein (MTP) (96). Targeting miR-122 in an animal model has demonstrated a significant reduction in cholesterol and triglyceride levels. However, this effect was overshadowed by the increased risk of hepatic cancer and fibrosis associated with miR-122 deletion (97)**—figure 1**. Besides, in animal model inhibition of miR-33 has shown to modify VLDL-C and triglyceride biosynthesis, and anti-miR-33 has significantly reduced VLDL-C and triglyceride (98). Expression of miR-33 shown to has a control on ABCA1 and ABCG1 through activation of SREBP-2, and inhibition of miR-33 increases the hepatic expression of ABCA1 and subsequently increases HDL levels (91, 92). Furthermore, several miRNAs have demonstrated control over LDL-C metabolism. For instance, inhibition of miR-128-1, miR-185 and miR-148a has markedly reduced LDL-C levels (99-101). Furthermore, the expression of miR-148a has shown to alter the blood levels of LDL-C by targeting 3'UTR of the LDLR and other genes vital for lipid metabolisms such as ABCA1, AMPK, PGC1α and SIK1. Additionally, miR-148a expression increases HDL-C levels by regulating ABCA1 expression in the liver (99, 100, 102). miR-130 and miR-143 are strongly linked to adipogenesis, the expression of miR-143 is upregulated in the obese animal model, and inhibition of miR-143 reduced insulin activated AKT (103). Moreover, overexpression of miR-130a inhibits adipocytes differentiation by suppressing PPAR-γ activity (104). Conversely, expression of miR-375 shown to induce adipogenesis by increasing PPAR-γ, C/EBP-α and promoting 3T3-L1 (105). A study found that the expression of miR-103 and miR-27b is significantly higher in women with PCOS compared to

women without PCOS (21). The expression of miR-23a and miR-23b has been positively correlated with body mass index (BMI) despite its lower level in women with PCOS (106). However, on the other hand, miR-199a-5p and miR-199a-3p were negatively correlated with waist/hip ratio and BMI (69). These findings indicated the strong association between miRNAs, obesity and dyslipidaemia and laid down its potentiality as a therapeutic target in the management of the metabolic aspects of PCOS.

miRNAs as a potential therapeutic target in PCOS

According to the current guidelines, strategies for management of PCOS are merely focused on alleviating symptoms and improve the prognostic outcomes. Various pharmacological approaches are used including insulin sensitising agents which enhance the insulin sensitivity and subsequently reduce IR, fertility treatment such as letrozole and clomiphene citrate to induce ovulation, anti-androgen therapies for the treatment of high androgen-related symptoms (i.e. hirsutism and acne) and oral contraceptives to regulate the menstrual cycle. Insulin resistance (IR) is a main pathological feature of PCOS and improving insulin sensitivity might facilitate glucose metabolism, reduces androgen levels and augments fertility.

Metformin is a drug that has been used for decades in the management of PCOS. It improves insulin sensitivity, impaired glucose tolerance (IGT) and consequently reduces androgen levels in women with PCOS (107, 108). Even though, its effect on induction of ovulation and improving fertility in women with PCOS still debatable, there is a considerable amount of evidence showed it has a significant impact in improving the rate of ovulation and the outcome of pregnancy (109-112). Recently, miRNAs have also attracted considerable interest as a potential target for therapeutic and prognosis of PCOS.

In a study; metformin administration decreased the expression of the pancreatic cancer stem cells (CSC) markers by increases the expression of miR-26a (113). Furthermore, metformin also downregulates miR-221 and miR-222 that promote intimal thickness in patients with T2DM (114).**Figure1**. Even more, evidence suggests that treatment with metformin upregulates DICER1 and enhances the stability of DICER1 mRNA and permitting DICER1 to accumulate, which may offer a new therapeutic approach for age-related health problems (115). Recently, the incretins-based treatment, including glucagon-like peptide 1 agonist receptor agonist (GLP-1 RA) and dipeptidyl peptidase-4 (DPP-4) inhibitors, have inspired researcher to examine its potential benefits for managing the metabolic aspect of PCOS. A recent study has demonstrated that overexpression of miR-155-5p and miR-33 stimulate insulin secretion by increasing the expression of GLP-1 on the β-cells of the pancreas (116). On the other hand, miR-197, miR-6356, miR-1197-3p, miR-875-5P and miR-6763 inhibit the incretin expression and therefore, reduce insulin secretion (117). GLP-1 RA increases the expression of miR-27a, miR-192, miR-132 but reduces miR-375 and miR-23 expression, which has a significant glycaemic effect by stimulating insulin secretion and thus, lowering blood glucose (117). Therefore, with the currently growing evidence on incretins-based therapies for T2DM management, and the newly emerging evidence about its influence on miRNAs expression. The incretins-miRNAs pathway might be a potential therapeutic target for PCOS; however, it is still a fertile ground for further scientific research.

The metabolic comorbidities associated with PCOS are mostly due to the vicious cycle between IR and high androgen levels. Therefore, the treatment approach targeting excess androgen levels can significantly improve the clinical manifestations associated with PCOS.

Anti-androgen therapies are used to treat symptoms of high androgen levels, such as hirsutism and acne. miR-212 and miR-199 have shown to modulate androgen receptor (AR) and enhances its production by targeting CYP19A1(118). Furthermore, miR-155 is correlated negatively with A4 concentration (71). miR-838-p3, miR-9563a-p3 and miR-9563-p5 are targeting ACO32, GDL73 and MFPA a transcript that regulate the long-chain fatty acid synthesis, lipid transport and metabolism (119).

Additionally, miR-27b also plays an essential role in regulating fatty acid and cholesterol metabolism (102), and miR-155 has a significant role in monitoring the effectiveness of anti-androgen therapy (71). These potential effects of miRNAs on androgen hormone might have a substantial role in improving PCOS related symptoms by augmenting anti-androgen treatments.

Conclusion and future direction

In conclusion, the diagnosis and the treatment of PCOS have emerged as one of the most significant challenges faced by clinicians and healthcare professionals. Over the last few years, several promising studies have been focused on the characterisation and identification of various miRNAs. Some PCOS-associated miRNAs are abundantly expressed in the ovaries, skeletal muscles, adipose tissues, and the pancreas. They regulate the follicular development and maturation, steroid hormone synthesis, adipogenesis, insulin signalling pathway. Given all these, miRNAs could potentially be clinical biomarkers for the diagnosis of PCOS and a therapeutic target in the treatment of PCOS. The potential miRNAs based therapeutic options will provide a new horizon and a compelling alternative for the treatment of PCOS and its related metabolic complications.

Limitation of miRNAs applied in clinical practice

There are apparent promises, hopes, enthusiasm and significant efforts to promote miRNAs-based products. However, despite the advances in the diagnostic field and the thousands of scientific research in this area, miRNAs as a potential diagnostic tool is still in their infancy. On the other hands, despite the potentials, miRNAs-based therapeutics are yet to be developed. Therefore, the development of commercially available miRNAs-based diagnostics and therapeutic tools is a long way to go.

Author contributions

M.A; participate with the conception, study design, drafting the manuscript, H.D; involved with the study design, critical revision, editing and approval of the final draft of the study, S.L.A; contributed with the study design, critical review, editing and the support of the final draft, T.S; contributed with the conception, study design, critical revision, editing and the final approval of the manuscript.

References

1. 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(6):2745-9.
2. Nandi A, Chen Z, Patel R, Poretsky L. Polycystic ovary syndrome. Endocrinol Metab Clin North Am. 2014;43(1):123-47.
3. Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19-25.
4. Christakou C, Diamanti-Kandarakis E. Polycystic ovary syndrome--phenotypes and diagnosis. Scand J Clin Lab Invest Suppl. 2014;244:18-22; discussion 1.
5. McDonnell R, Hart RJ. Pregnancy-related outcomes for women with polycystic ovary syndrome. Womens Health (Lond). 2017;13(3):89-97.
6. Witchel SF, Oberfield SE, Pena AS. Polycystic Ovary Syndrome: Pathophysiology, Presentation, and Treatment With Emphasis on Adolescent Girls. J Endocr Soc. 2019;3(8):1545-73.

7. Ambros V. microRNAs: tiny regulators with great potential. Cell. 2001;107(7):823-6.
8. Flynt AS, Lai EC. Biological principles of microRNA-mediated regulation: shared themes amid diversity. Nat Rev Genet. 2008;9(11):831-42.
9. Lawrie CH, Saunders NJ, Soneji S, Palazzo S, Dunlop HM, Cooper CD, et al. MicroRNA expression in lymphocyte development and malignancy. Leukemia. 2008;22(7):1440-6.
10. Bar M, Wyman SK, Fritz BR, Qi J, Garg KS, Parkin RK, et al. MicroRNA discovery and profiling in human embryonic stem cells by deep sequencing of small RNA libraries. Stem Cells. 2008;26(10):2496-505.
11. Gallo A, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. PLoS One. 2012;7(3):e30679.
12. Hanson EK, Lubenow H, Ballantyne J. Identification of forensically relevant body fluids using a panel of differentially expressed microRNAs. Anal Biochem. 2009;387(2):303-14.
13. Ludwig N, Leidinger P, Becker K, Backes C, Fehlmann T, Pallasch C, et al. Distribution of miRNA expression across human tissues. Nucleic Acids Res. 2016;44(8):3865-77.
14. Xue Y, Lv J, Xu P, Gu L, Cao J, Xu L, et al. Identification of microRNAs and genes associated with hyperandrogenism in the follicular fluid of women with polycystic ovary syndrome. J Cell Biochem. 2018;119(5):3913-21.
15. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids--the mix of hormones and biomarkers. Nat Rev Clin Oncol. 2011;8(8):467-77.
16. Mari-Alexandre J, Barcelo-Molina M, Belmonte-Lopez E, Garcia-Oms J, Estelles A, Braza-Boils A, et al. Micro-RNA profile and proteins in peritoneal fluid from women with endometriosis: their relationship with sterility. Fertil Steril. 2018;109(4):675-84 e2.
17. Romakina VV, Zhirov IV, Nasonova SN, Zaseeva AV, Kochetov AG, Liang OV, et al. [MicroRNAs as Biomarkers of Cardiovascular Diseases]. Kardiologiia. 2018(1):66-71.
18. Guay C, Regazzi R. Circulating microRNAs as novel biomarkers for diabetes mellitus. Nat Rev Endocrinol. 2013;9(9):513-21.
19. Long W, Zhao C, Ji C, Ding H, Cui Y, Guo X, et al. Characterization of serum microRNAs profile of PCOS and identification of novel non-invasive biomarkers. Cell Physiol Biochem. 2014;33(5):1304-15.
20. Chang RJ, Cook-Andersen H. Disordered follicle development. Mol Cell Endocrinol. 2013;373(1-2):51-60.
21. 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(11):E1835-44.
22. 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(1):49-56.
23. Bhartiya D, James K. Very small embryonic-like stem cells (VSELs) in adult mouse uterine perimetrium and myometrium. J Ovarian Res. 2017;10(1):29.
24. Sontakke SD, Mohammed BT, McNeilly AS, Donadeu FX. Characterization of microRNAs differentially expressed during bovine follicle development. Reproduction. 2014;148(3):271-83.
25. Chen B, Xu P, Wang J, Zhang C. The role of MiRNA in polycystic ovary syndrome (PCOS). Gene. 2019;706:91-6.
26. Liu J, Li X, Yao Y, Li Q, Pan Z, Li Q. miR-1275 controls granulosa cell apoptosis and estradiol synthesis by impairing LRH-1/CYP19A1 axis. Biochim Biophys Acta Gene Regul Mech. 2018;1861(3):246-57.
27. Sirotkin AV, Kisova G, Brenaut P, Ovcharenko D, Grossmann R, Mlyncek M. Involvement of microRNA Mir15a in control of human ovarian granulosa cell proliferation, apoptosis, steroidogenesis, and response to FSH. Microrna. 2014;3(1):29-36.
28. Nie M, Yu S, Peng S, Fang Y, Wang H, Yang X. miR-23a and miR-27a promote human granulosa cell apoptosis by targeting SMAD5. Biol Reprod. 2015;93(4):98.

29. Yang X, Zhou Y, Peng S, Wu L, Lin HY, Wang S, et al. Differentially expressed plasma microRNAs in premature ovarian failure patients and the potential regulatory function of mir-23a in granulosa cell apoptosis. Reproduction. 2012;144(2):235-44.
30. Jiang L, Huang J, Li L, Chen Y, Chen X, Zhao X, et al. MicroRNA-93 promotes ovarian granulosa cells proliferation through targeting CDKN1A in polycystic ovarian syndrome. J Clin Endocrinol Metab. 2015;100(5):E729-38.
31. Roush S, Slack FJ. The let-7 family of microRNAs. Trends Cell Biol. 2008;18(10):505-16.
32. Cao R, Wu W, Zhou X, Liu K, Li B, Huang X, et al. Let-7g induces granulosa cell apoptosis by targeting MAP3K1 in the porcine ovary. Int J Biochem Cell Biol. 2015;68:148-57.
33. Guo Y, Sun J, Lai D. Role of microRNAs in premature ovarian insufficiency. Reprod Biol Endocrinol. 2017;15(1):38.
34. Yang S, Wang S, Luo A, Ding T, Lai Z, Shen W, 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(5):126.
35. 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(6141):71-3.
36. Hossain MM, Cao M, Wang Q, Kim JY, Schellander K, Tesfaye D, et al. Altered expression of miRNAs in a dihydrotestosterone-induced rat PCOS model. J Ovarian Res. 2013;6(1):36.
37. Zhang H, Jiang X, Zhang Y, Xu B, Hua J, Ma T, et al. microRNA 376a regulates follicle assembly by targeting Pcna in fetal and neonatal mouse ovaries. Reproduction. 2014;148(1):43-54.
38. Zhang J, Ji X, Zhou D, Li Y, Lin J, Liu J, et al. MiR-143 is critical for the formation of primordial follicles in mice. Frontiers in bioscience : a journal and virtual library. 2013;18:588-97.
39. Yao G, Yin M, Lian J, Tian H, Liu L, Li X, et al. MicroRNA-224 is involved in transforming growth factor-beta-mediated mouse granulosa cell proliferation and granulosa cell function by targeting Smad4. Mol Endocrinol. 2010;24(3):540-51.
40. Yao G, Liang M, Liang N, Yin M, Lu M, Lian J, et al. MicroRNA-224 is involved in the regulation of mouse cumulus expansion by targeting Ptx3. Mol Cell Endocrinol. 2014;382(1):244-53.
41. Huang X, Hao C, Shen X, Zhang Y, Liu X. RUNX2, GPX3 and PTX3 gene expression profiling in cumulus cells are reflective oocyte/embryo competence and potentially reliable predictors of embryo developmental competence in PCOS patients. Reprod Biol Endocrinol. 2013;11:109.
42. Moreno JM, Nunez MJ, Quinonero A, Martinez S, de la Orden M, Simon C, et al. Follicular fluid and mural granulosa cells microRNA profiles vary in in vitro fertilization patients depending on their age and oocyte maturation stage. Fertil Steril. 2015;104(4):1037-46 e1.
43. Sang Q, Yao Z, Wang H, Feng R, Wang H, Zhao X, et al. 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(7):3068-79.
44. Revelli A, Delle Piane L, Casano S, Molinari E, Massobrio M, Rinaudo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. Reprod Biol Endocrinol. 2009;7:40.
45. Chen Z, Ou H, Wu H, Wu P, Mo Z. Role of microRNA in the Pathogenesis of Polycystic Ovary Syndrome. DNA Cell Biol. 2019;38(8):754-62.
46. Butler AE, Ramachandran V, Hayat S, Dargham SR, Cunningham TK, Benurwar M, et al. Expression of microRNA in follicular fluid in women with and without PCOS. Sci Rep. 2019;9(1):16306.
47. Sathyapalan T, David R, Gooderham NJ, Atkin SL. Increased expression of circulating miRNA-93 in women with polycystic ovary syndrome may represent a novel, non-invasive biomarker for diagnosis. Sci Rep. 2015;5:16890.
48. Sorensen AE, Wissing ML, Englund AL, Dalgaard LT. MicroRNA Species in Follicular Fluid Associating With Polycystic Ovary Syndrome and Related Intermediary Phenotypes. J Clin Endocrinol Metab. 2016;101(4):1579-89.

49. Scalici E, Traver S, Mullet T, Molinari N, Ferrieres A, Brunet C, et al. Circulating microRNAs in follicular fluid, powerful tools to explore in vitro fertilization process. Sci Rep. 2016;6:24976.
50. Lin L, Du T, Huang J, Huang LL, Yang DZ. Identification of differentially expressed microRNAs in the ovary of polycystic ovary syndrome with hyperandrogenism and insulin resistance. Chin Med J (Engl). 2015;128(2):169-74.
51. Roth LW, McCallie B, Alvero R, Schoolcraft WB, Minjarez D, Katz-Jaffe MG. Altered microRNA and gene expression in the follicular fluid of women with polycystic ovary syndrome. J Assist Reprod Genet. 2014;31(3):355-62.
52. Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, et al. MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 2004;23(20):4051-60.
53. Hossain MM, Sohel MM, Schellander K, Tesfaye D. Characterization and importance of microRNAs in mammalian gonadal functions. Cell Tissue Res. 2012;349(3):679-90.
54. McCallie B, Schoolcraft WB, Katz-Jaffe MG. Aberration of blastocyst microRNA expression is associated with human infertility. Fertil Steril. 2010;93(7):2374-82.
55. Medeiros LA, Dennis LM, Gill ME, Houbaviy H, Markoulaki S, Fu D, et al. Mir-290-295 deficiency in mice results in partially penetrant embryonic lethality and germ cell defects. Proc Natl Acad Sci U S A. 2011;108(34):14163-8.
56. Kim YJ, Ku SY, Kim YY, Liu HC, Chi SW, Kim SH, et al. MicroRNAs transfected into granulosa cells may regulate oocyte meiotic competence during in vitro maturation of mouse follicles. Hum Reprod. 2013;28(11):3050-61.
57. McCallie BR, Parks JC, Strieby AL, Schoolcraft WB, Katz-Jaffe MG. Human blastocysts exhibit unique microrna profiles in relation to maternal age and chromosome constitution. J Assist Reprod Genet. 2014;31(7):913-9.
58. Kamalidehghan B, Habibi M, Afjeh SS, Shoai M, Alidoost S, Almasi Ghale R, et al. The Importance of Small Non-Coding RNAs in Human Reproduction: A Review Article. Appl Clin Genet. 2020;13:1-11.
59. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. Endocr Rev. 2016;37(5):467-520.
60. Rodriguez Paris V, Bertoldo MJ. The Mechanism of Androgen Actions in PCOS Etiology. Med Sci (Basel). 2019;7(9).
61. Kiddy DS, Sharp PS, White DM, Scanlon MF, Mason HD, Bray CS, et al. Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 263 consecutive cases. Clin Endocrinol (Oxf). 1990;32(2):213-20.
62. Kempna P, Marti N, Udhane S, Fluck CE. Regulation of androgen biosynthesis - A short review and preliminary results from the hyperandrogenic starvation NCI-H295R cell model. Mol Cell Endocrinol. 2015;408:124-32.
63. Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nat Rev Endocrinol. 2018;14(5):270-84.
64. Walters KA. Role of androgens in normal and pathological ovarian function. Reproduction. 2015;149(4):R193-218.
65. Apparao KB, Lovely LP, Gui Y, Lininger RA, Lessey BA. Elevated endometrial androgen receptor expression in women with polycystic ovarian syndrome. Biol Reprod. 2002;66(2):297-304.
66. Hillier SG, Tetsuka M, Fraser HM. Location and developmental regulation of androgen receptor in primate ovary. Hum Reprod. 1997;12(1):107-11.
67. Troppmann B, Kossack N, Nordhoff V, Schuring AN, Gromoll J. MicroRNA miR-513a-3p acts as a co-regulator of luteinizing hormone/chorionic gonadotropin receptor gene expression in human granulosa cells. Mol Cell Endocrinol. 2014;390(1-2):65-72.
68. Sirotkin AV, Ovcharenko D, Grossmann R, Laukova M, Mlyncek M. Identification of microRNAs controlling human ovarian cell steroidogenesis via a genome-scale screen. J Cell Physiol. 2009;219(2):415-20.

69. Murri M, Insenser M, Fernandez-Duran E, San-Millan JL, Luque-Ramirez M, Escobar-Morreale HF. Non-targeted profiling of circulating microRNAs in women with polycystic ovary syndrome (PCOS): effects of obesity and sex hormones. Metabolism. 2018;86:49-60.
70. Sorensen AE, Wissing ML, Salo S, Englund AL, Dalgaard LT. MicroRNAs Related to Polycystic Ovary Syndrome (PCOS). Genes (Basel). 2014;5(3):684-708.
71. Arancio W, Calogero Amato M, Magliozzo M, Pizzolanti G, Vesco R, Giordano C. Serum miRNAs in women affected by hyperandrogenic polycystic ovary syndrome: the potential role of miR-155 as a biomarker for monitoring the estroprogestinic treatment. Gynecol Endocrinol. 2018;34(8):704-8.
72. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev. 1994;15(3):342-55.
73. Xu S, Linher-Melville K, Yang BB, Wu D, Li J. Micro-RNA378 (miR-378) regulates ovarian estradiol production by targeting aromatase. Endocrinology. 2011;152(10):3941-51.
74. Zhang Q, Sun H, Jiang Y, Ding L, Wu S, Fang T, et al. MicroRNA-181a suppresses mouse granulosa cell proliferation by targeting activin receptor IIA. PLoS One. 2013;8(3):e59667.
75. Dai A, Sun H, Fang T, Zhang Q, Wu S, Jiang Y, et al. MicroRNA-133b stimulates ovarian estradiol synthesis by targeting Foxl2. FEBS Lett. 2013;587(15):2474-82.
76. Uda M, Ottolenghi C, Crisponi L, Garcia JE, Deiana M, Kimber W, et al. Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. Hum Mol Genet. 2004;13(11):1171-81.
77. Diamanti-Kandarakis E. Insulin resistance in PCOS. Endocrine. 2006;30(1):13-7.
78. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012;33(6):981-1030.
79. Plymate SR, Matej LA, Jones RE, Friedl KE. Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. J Clin Endocrinol Metab. 1988;67(3):460-4.
80. Zhang G, Garmey JC, Veldhuis JD. Interactive stimulation by luteinizing hormone and insulin of the steroidogenic acute regulatory (StAR) protein and 17alpha-hydroxylase/17,20-lyase (CYP17) genes in porcine theca cells. Endocrinology. 2000;141(8):2735-42.
81. Ling HY, Ou HS, Feng SD, Zhang XY, Tuo QH, Chen LX, et al. CHANGES IN microRNA (miR) profile and effects of miR-320 in insulin-resistant 3T3-L1 adipocytes. Clin Exp Pharmacol Physiol. 2009;36(9):e32-9.
82. Mao Y, Mohan R, Zhang S, Tang X. MicroRNAs as pharmacological targets in diabetes. Pharmacol Res. 2013;75:37-47.
83. Jiang L, Huang J, Chen Y, Yang Y, Li R, Li Y, et al. Identification of several circulating microRNAs from a genome-wide circulating microRNA expression profile as potential biomarkers for impaired glucose metabolism in polycystic ovarian syndrome. Endocrine. 2016;53(1):280-90.
84. Chen YH, Heneidi S, Lee JM, Layman LC, Stepp DW, Gamboa GM, et al. miRNA-93 inhibits GLUT4 and is overexpressed in adipose tissue of polycystic ovary syndrome patients and women with insulin resistance. Diabetes. 2013;62(7):2278-86.
85. Yang Y, Jiang H, Xiao L, Yang X. MicroRNA-33b-5p is overexpressed and inhibits GLUT4 by targeting HMGA2 in polycystic ovarian syndrome: An in vivo and in vitro study. Oncol Rep. 2018;39(6):3073-85.
86. Chakraborty C, Doss CG, Bandyopadhyay S, Agoramoorthy G. Influence of miRNA in insulin signaling pathway and insulin resistance: micro-molecules with a major role in type-2 diabetes. Wiley Interdiscip Rev RNA. 2014;5(5):697-712.
87. Shi L, Liu S, Zhao W, Shi J. miR-483-5p and miR-486-5p are down-regulated in cumulus cells of metaphase II oocytes from women with polycystic ovary syndrome. Reprod Biomed Online. 2015;31(4):565-72.

88. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. Am J Med. 2001;111(8):607-13.
89. Berneis K, Rizzo M, Lazzarini V, Fruzzetti F, Carmina E. Atherogenic lipoprotein phenotype and low-density lipoproteins size and subclasses in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92(1):186-9.
90. Wang M, Zhao D, Xu L, Guo W, Nie L, Lei Y, et al. Role of PCSK9 in lipid metabolic disorders and ovarian dysfunction in polycystic ovary syndrome. Metabolism. 2019;94:47-58.
91. Rayner KJ, Suarez Y, Davalos A, Parathath S, Fitzgerald ML, Tamehiro N, et al. MiR-33 contributes to the regulation of cholesterol homeostasis. Science. 2010;328(5985):1570-3.
92. Najafi-Shoushtari SH, Kristo F, Li Y, Shioda T, Cohen DE, Gerszten RE, et al. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. Science. 2010;328(5985):1566-9.
93. Marquart TJ, Allen RM, Ory DS, Baldan A. miR-33 links SREBP-2 induction to repression of sterol transporters. Proc Natl Acad Sci U S A. 2010;107(27):12228-32.
94. Li T, Francl JM, Boehme S, Chiang JY. Regulation of cholesterol and bile acid homeostasis by the cholesterol 7alpha-hydroxylase/steroid response element-binding protein 2/microRNA-33a axis in mice. Hepatology. 2013;58(3):1111-21.
95. Allen RM, Marquart TJ, Albert CJ, Suchy FJ, Wang DQ, Ananthanarayanan M, et al. miR-33 controls the expression of biliary transporters, and mediates statin- and diet-induced hepatotoxicity. EMBO Mol Med. 2012;4(9):882-95.
96. Soh J, Iqbal J, Queiroz J, Fernandez-Hernando C, Hussain MM. MicroRNA-30c reduces hyperlipidemia and atherosclerosis in mice by decreasing lipid synthesis and lipoprotein secretion. Nature Medicine. 2013;19(7):892-900.
97. Hsu SH, Wang B, Kota J, Yu J, Costinean S, Kutay H, et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. J Clin Invest. 2012;122(8):2871-83.
98. Rayner KJ, Esau CC, Hussain FN, McDaniel AL, Marshall SM, van Gils JM, et al. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. Nature. 2011;478(7369):404-7.
99. Wagschal A, Najafi-Shoushtari SH, Wang L, Goedeke L, Sinha S, deLemos AS, et al. Genome-wide identification of microRNAs regulating cholesterol and triglyceride homeostasis. Nat Med. 2015;21(11):1290-7.
100. Goedeke L, Rotllan N, Canfran-Duque A, Aranda JF, Ramirez CM, Araldi E, et al. MicroRNA-148a regulates LDL receptor and ABCA1 expression to control circulating lipoprotein levels. Nat Med. 2015;21(11):1280-9.
101. Iacomino G, Siani A. Role of microRNAs in obesity and obesity-related diseases. Genes Nutr. 2017;12:23.
102. Fernandez-Hernando C, Suarez Y, Rayner KJ, Moore KJ. MicroRNAs in lipid metabolism. Curr Opin Lipidol. 2011;22(2):86-92.
103. Jordan SD, Kruger M, Willmes DM, Redemann N, Wunderlich FT, Bronneke HS, et al. Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. Nat Cell Biol. 2011;13(4):434-46.
104. Lee EK, Lee MJ, Abdelmohsen K, Kim W, Kim MM, Srikantan S, et al. miR-130 suppresses adipogenesis by inhibiting peroxisome proliferator-activated receptor gamma expression. Mol Cell Biol. 2011;31(4):626-38.
105. Ling HY, Wen GB, Feng SD, Tuo QH, Ou HS, Yao CH, et al. MicroRNA-375 promotes 3T3-L1 adipocyte differentiation through modulation of extracellular signal-regulated kinase signalling. Clin Exp Pharmacol Physiol. 2011;38(4):239-46.
106. Xiong W, Lin Y, Xu L, Tamadon A, Zou S, Tian F, et al. Circulatory microRNA 23a and microRNA 23b and polycystic ovary syndrome (PCOS): the effects of body mass index and sex hormones in an Eastern Han Chinese population. J Ovarian Res. 2017;10(1):10.

107. Lord JM, Flight IH, Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ. 2003;327(7421):951-3.
108. Lashen H. Role of metformin in the management of polycystic ovary syndrome. Ther Adv Endocrinol Metab. 2010;1(3):117-28.
109. Nestler JE. Metformin in the treatment of infertility in polycystic ovarian syndrome: an alternative perspective. Fertil Steril. 2008;90(1):14-6.
110. Baran S, Api M, Goksedef BP, Cetin A. Comparison of metformin and clomiphene citrate therapy for induction of ovulation in the polycystic ovary syndrome. Arch Gynecol Obstet. 2010;282(4):439-43.
111. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engl J Med. 1998;338(26):1876-80.
112. Oppelt PG, Mueller A, Jentsch K, Kronawitter D, Reissmann C, Dittrich R, et al. The effect of metformin treatment for 2 years without caloric restriction on endocrine and metabolic parameters in women with polycystic ovary syndrome. Exp Clin Endocrinol Diabetes. 2010;118(9):633-7.
113. Bao B, Wang Z, Ali S, Ahmad A, Azmi AS, Sarkar SH, et al. Metformin inhibits cell proliferation, migration and invasion by attenuating CSC function mediated by deregulating miRNAs in pancreatic cancer cells. Cancer Prev Res (Phila). 2012;5(3):355-64.
114. Coleman CB, Lightell DJ, Jr., Moss SC, Bates M, Parrino PE, Woods TC. Elevation of miR-221 and -222 in the internal mammary arteries of diabetic subjects and normalization with metformin. Mol Cell Endocrinol. 2013;374(1-2):125-9.
115. Noren Hooten N, Martin-Montalvo A, Dluzen DF, Zhang Y, Bernier M, Zonderman AB, et al. Metformin-mediated increase in DICER1 regulates microRNA expression and cellular senescence. Aging Cell. 2016;15(3):572-81.
116. Capuani B, Pacifici F, Della-Morte D, Lauro D. Glucagon Like Peptide 1 and MicroRNA in Metabolic Diseases: Focusing on GLP1 Action on miRNAs. Front Endocrinol (Lausanne). 2018;9:719.
117. Radbakhsh S, Sathyapalan T, Banach M, Sahebkar A. Incretins and microRNAs: Interactions and physiological relevance. Pharmacol Res. 2020;153:104662.
118. Sorensen AE, Udesen PB, Wissing ML, Englund ALM, Dalgaard LT. MicroRNAs related to androgen metabolism and polycystic ovary syndrome. Chem Biol Interact. 2016;259(Pt A):8-16.
119. Wang Z, Qiao Y, Zhang J, Shi W, Zhang J. Genome wide identification of microRNAs involved in fatty acid and lipid metabolism of Brassica napus by small RNA and degradome sequencing. Gene. 2017;619:61-70.

Table1: miRNAs detected in PCOS and their proposed functions

miRNAs Detected in PCOS	Detected in tissues	Proposed functions	References
miR-376 Increased expression in PCOS	GCs	Increase GCs proliferation by Increase expression of PCNA	(37)
miR-224 Increased expression in PCOS	GCs	Increase fertilisation by increase PTX-3 expression in GCs	(41)
miR-182 /miR-15a Decreased expression in PCOS	GCs	Regulate steroidogenesis and promote GCs proliferation and apoptosis	(27, 36)
miR-132/miR-320 Decreased expression in PCOS	GCs	Regulate steroidogenesis	(43)
miR-320 Increased expression in PCOS	Adipocytes cells	Increase IR by downregulate GLUT4	(81)
miR-33b-5p Increased expression in PCOS	GCs	Negatively correlate with GLUT4, SREBF1 and HMGA2 and promote IR	(85)
miR-27b Increased expression in PCOS	Blood	Induce adipogenesis by increasing PPAR-γ and C/EBP-α	(21)
miR-103/ miR-155 Increased expression in PCOS and correlate with increased testosterone	Blood/GCs	Induce progesterone release and inhibit oestradiol release	(69)
miR-155 Increased expression in PCOS	GCs	Inhibit testosterone release by inhibiting PCNA	(71)

GCs; granulosa cells**, PCNA;** proliferating cell nuclear antigen protein, **PTX-3;** Pentraxin 3, **GLUT4;** Glucose transporter 4, **SREBF1;** sterol regulatory element-binding protein,**HMGA2;** high mobility group A2**,IR;** insulin resistance, **PPAR-γ;** peroxisome proliferator activating factor-γ, **C/EBP-α;** c/enhancer-binding protein-α. These miRNAs are just an example, not the full list.

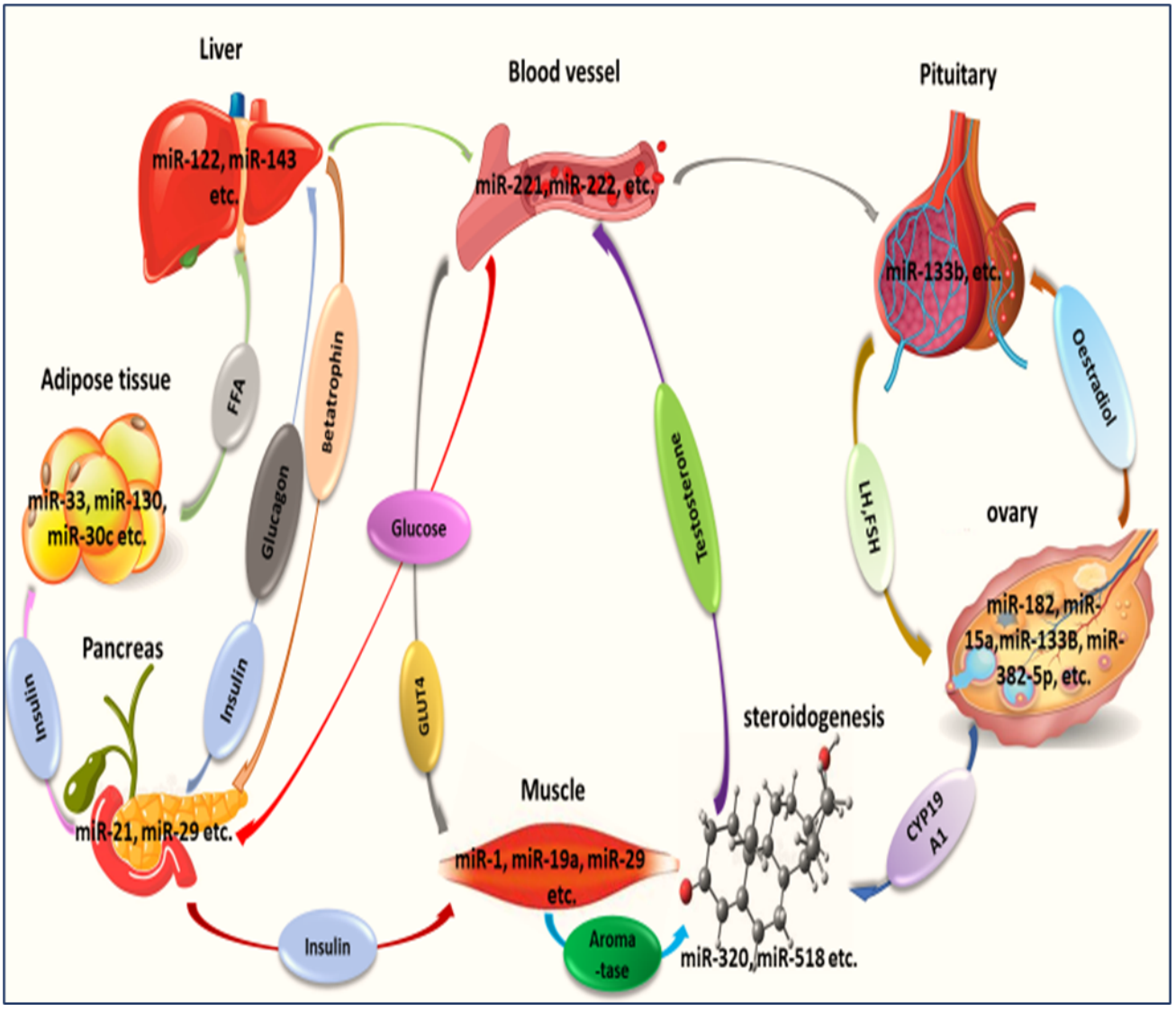

Figure 1: The expression of miRNAs in different organs and their cross talk in regulating follicular development, follicular maturation, steroidogenesis, glucose metabolism, insulin resistance, adipogenesis and lipid metabolism.

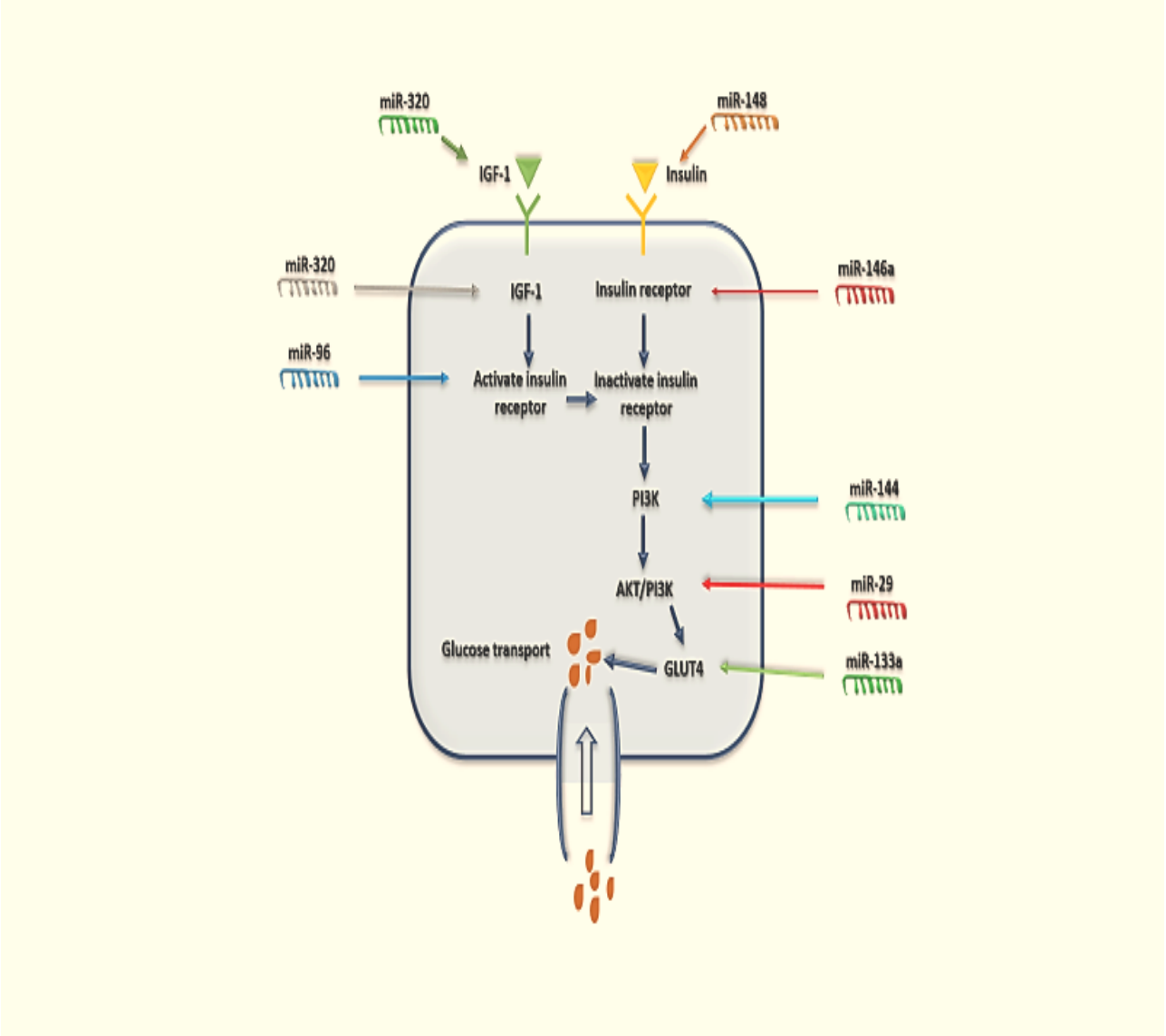

Figure2: Role of miRNAs in the insulin signalling pathway and insulin resistance.