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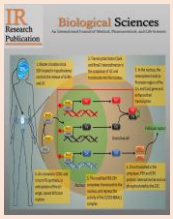

Importance of biological clock epigenetics in the manifestation of polycystic ovary syndrome

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ABSTRACT

In recent times, the incidence of polycystic ovary syndrome (PCOS) increases at an alarming rate. It is ascribed to the metabolic and endocrine imbalance, which subsequently adversely impacts the fertility of women. Growing evidence indicates the crucial role of circadian biology in the development and progression of PCOS. Here, we describe the role of epigenetic machinery related to circadian genes (*Clock, Bmal, Per,* and *Cry*) on insulin resistance and hyperandrogenism components of the PCOS. We comprehensively discuss the interconnection between the genetics of circadian rhythm and PCOS pathophysiology. Deeper insights into the epigenetic changes of circadian genes may open new routes for the epigenetic regulation of ovarian physiology and PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine and metabolic ailment in women characterized by hyperandrogenism, ovulatory disorder, polycystic ovarian morphology (PCOM), and hyperinsulinemia (1). In premenopausal women, PCOS occurrence ranges from 6~20%, indicating the endocrine and metabolic origin of the sickness at reproductive age of women (2,3). The infertility rate is 70-80% in women with PCOS (4). Multiple studies reported that women with PCOS have a 2.7-fold increased risk for developing endometrial cancer. However, the risk of ovarian and breast cancer remains unchanged (5). The risk factors for PCOS include genetic changes, insulin resistance (IR), hyperinsulinemia, hyperandrogenism, ovarian follicular maturation arrest, gonadotropic derangements, and obesity (1,3). The daily 24 h sleep-wake cycle is maintained by circadian rhythm, involved in directing various endogenous physiological, metabolic functions, and genetic expressions (6). The circadian rhythm controls the metabolic activity, as distressed circadian rhythm is connected with various metabolic syndromes like diabetes, insulin resistance, and obesity (7). Sleep disturbances and sleep apnoea increase the PCOS incidence by two-fold (8). The two well-known biomarkers of the circadian cycle are cortisol and melatonin, as cortisol peaks in the early morning and melatonin levels rise in the night. Interestingly,

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Abbreviations: *AR: Androgen receptor; Bmal: Brain and muscle ARNT-like protein; BMI: Body mass index; CK: Casein kinases; Clock: Circadian locomotor output cycles kaput; CREB: cAMP response element binding protein; Cry: Cryptochrome; DAX: Nuclear receptor subfamily; DNMT: DNA methyltransferase; EPHX: Epoxide hydrolase; ESR: Estrogen receptor; EZH: Zeste homolog ; FOXL: Forkhead Box L; FSH: Follicle stimulating hormone; GLUT: Glucose transporter type; GnRH: Gonadotropin-releasing hormone; HAT: Histone acetyltransferases; HDAC: Histone deacetylase; HDM: Histone demethylase; HFD: High-fat diet; HGC: Human chorionic gonadotropin; HMGA: high-mobility group AT; HMT: Histone methyl transferase; HOMA-IR: Homeostatic model assessment for insulin resistance; HSD3B: 3-beta-hydroxysteroid dehydrogenase; IER: Immediate early response; IGF1R: Insulin like growth factor 1 receptor; INSR: Insulin receptor; IR: Insulin resistance; IRS: Insulin receptor substrate; LH: Luteinising hormone ; MeDIP: Methylated DNA immunoprecipitation; miRNA: MicroRNA; MTNR: Melatonin receptor; NAD: Nicotinamide-adenine dinucleotide; NCOR: nuclear corepressor; NPAS: Neuronal Per-Arnt-Sim domain-containing protein; ORP: Oxysterol-binding protein-related protein; PCOM: Polycystic ovarian morphology; PCOS: Polycystic ovary syndrome; Per: Period ; PPAR: peroxisome proliferator-initiated receptor gamma; PTEN: Phosphatase and tensin homolog; SCN: Suprachiasmatic nuclei; SF: Steroidogenic factor; SNP: Single-nucleotide polymorphism; SRD5A: 3-oxo-5α-steroid 4-dehydrogenase; STAR: Steroidogenic acute regulatory protein; T2DM:Type 2 diabetes mellitus*

women with PCOS showed an elevated level of melatonin in the morning, which indicates a disturbed circadian cycle. The rise of cortisol levels in PCOS subjects might indicate stress-mediated hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis (9). A typical component of PCOS is a disturbance of neuroendocrine and endocrine function, suggesting sporadic cycles and timing of ovarian steroid hormone discharge (10). Changes in the clock gene expression in the ovary may occur due to the hormonal imbalances associated with these disorders (11). Hyperandrogenemia prompts a dramatic change in the timing system, especially clock gene expression in the ovarian follicle and liver (12). This impact on the timing of ovarian clock gene expression may intensify the adverse effects of abundant androgen on key segments of the steroid biosynthetic and ovulatory response pathways (13). Dysfunctional epigenetic machinery has been noted as a pertinent factor in several diseases such as obesity and diabetes (14), and prostate cancer (15). PCOS, being a common denominator for both reproductive and metabolic abnormalities, may also be epigenetically regulated (16,17). Emerging studies suspected the role of circadian epigenetic in the development of PCOS (18–22). A higher level of global DNA methylation was reported in PCOS-overweight patients (23). miRNA is expressed differently in PCOS linked with several other mechanisms such as insulin signaling, inflammation, adipogenesis, and hyperandrogenemia (24). In PCOS, ovarian dysfunction with hyperandrogenism is associated with the alteration of HDAC3. Several PPAR agonists such as pioglitazone and rosiglitazone significantly mitigate hyperandrogenism and improve ovulation rates in PCOS women. Treatment with PPAR agonist also suggested resuming the normal ovarian function. Thus, downregulation of PPARγ1 expression in the granulosa cells (GCs) and induction of ovarian dysfunction in PCOS patients may be due to hyperandrogenism. The epigenetic alterations of PPARγ1 may lead to ovarian dysfunction induced by hyperandrogenism. However, since epigenetic changes in the circadian genes with respect to PCOS incidence are poorly discussed, herein we describe the interconnection of these two systems in the development and progression of PCOS.

Epigenetics

The altered epigenetic programming has been associated with various diseases, including diabetes (14) and prostate cancer (15). Since PCOS is concurrently seen with both reproductive and metabolic abnormalities (16,17), the epigenetic mechanisms may be critical in this phenomenon. Epigenetic changes are generally assessed by DNA methylation, histone modifications, and RNA-related silencing (25). Aberrations in the epigenome contribute to numerous ailments etiology, both prenatal and postnatal life (26).

DNA Methylation

DNA methylation can be presented as a biomarker for epigenetic modification. Epigenetic alteration by DNA methylation in the mammalian genome includes methyl group switch at the cytosine C5 position to produce 5-methylcytosine. Noteworthy, a massive number of DNMTs have been differentially methylated in adipose tissue of women with PCOS (27). The unaltered total methylation was reported in PCOS patients with the global DNA methylation examinations on peripheral blood leukocytes (28). In further studies, higher global methylated DNA levels in PCOS patients and also harbored a bounty of additional hypermethylated CpG locales. Thus, DNA methylation may enhance the function of a gene in PCOS ovary granulosa cells (23). The genome-wide methylated DNA immunoprecipitation (MeDIP) has been used to identify abnormally methylated genes in patients with PCOS. Seventy-nine genes were abnormally methylated in PCOS-non-IR versus PCOS-IR patients, and 40 genes were abnormally methylated in PCOS sufferers than healthy controls (28,29). Moreover, hyperandrogenism has been connected with the methylation status of genes indispensable for ordinary reproduction and ovarian capacity. For instance, a study carried out in hyperandrogenic females revealed 2 hypermethylated and 5

hypomethylated CpG sites in the PPAR□1 and nuclear corepressor 1 (NCOR1) promoter regions (30). In another study, Sang et al. (2014) determined the methylation degree of each CpG site in the promoter of epoxide hydrolase1 (EPHX1), 3-oxo-5α-steroid 4-dehydrogenase (SRD5A1), and CYP11A1 in 64 peripheral blood samples. They found a connection between methylation of the EPHX1 promoter with PCOS, proposed a key role of methylation in PCOS (31).

Histone modifications

Histone deacetylase (HDACs) and histone acetyltransferase (HATs) are key enzymes that influence DNA transcription (32). Chen and Fang (2018) revealed increased STAR production for the increased ovarian and adrenal androgen in PCOS patients (33). In PCOS ovaries, Jansen et al. (2004) identified down-regulated HDAC3 and increased PPAR□. Valproic acid, which acts as an HDAC-inhibitor and PPAR□ agonist, exhibits PCOS-like features such as hyperandrogenism and polycystic ovary (34,35). An increase in expression of HDAC6 is associated with the IR in PCOS through phosphatase and tensin homolog (PTEN) deacetylation. Tian et al. (2015) observed that Chinese natural remedy eases hyperandrogenism of PCOS rats via regulating PPAR□1 and HDAC3 expression in the ovaries of PCOS rats (36). Several studies manifested that IR can be reversed through HDAC inhibitors that ought to be a plausible strategy in the remedy of PCOS (37–39). Overweight is one of the predominant contributing components for PCOS (1). A study carried out by Xue et al. (2018) suggested that g9a/EHMT2, a histone methyltransferase, was markedly reduced in the liver of db/db mice and high-fat diet (HFD)-fed mice.

Non-coding RNA

miRNAs are regulatory small ssRNA molecules (21–25 nucleotides) involved in the post-transcriptional regulation of mRNAs by binding with the 3′-UTR of a target mRNA resulting in degradation or translational inhibition of the transcript (40,41). Any specific miRNA can modulate several target genes expression and function (42). Thus, they are involved in numerous physiological and pathological conditions, including cell proliferation, apoptosis, development, and carcinogenesis (43). Several studies demonstrated the significance of miRNAs in PCOS (22). A study carried out by Hossain et al. (2013) recommends that miRNAs are differentially regulated in hyperandrogenism, a situation possibly involved in the dysregulation of steroid hormone receptors and intra-ovarian factors, and that miRNAs may additionally be involved in the etiology of PCOS (44). miRNA expression in the blood and follicular fluid of PCOS patients involved in the disease phenotype's progression (45). Long et al. (2014) confirmed that miR-222, miR-146a, and miR-30c might additionally serve as novel biomarkers for PCOS diagnosis. A genome-wide association study recently concluded high-mobility group AT-hook 2 (Hmga2) and rab5b as PCOS candidate genes and are anticipated to be the target genes of miR-132 and miR-320. Sang et al. (2013) postulated that decrease miR-132 and miR-320 expression levels in the follicular fluid of PCOS sufferers might also influence Hmga2 and rab5b gene expression. They also observed that miR-132 and miR-320 are substantially related to PCOS, and the expression levels of both are lower in PCOS patients (46). Comparison of the expression profiles confirmed that miR-30a was markedly up-regulated, while miR-140 and let-7b had been substantially down-regulated in follicular fluid pools from patients with PCOS (n = 30). Consequently, down-regulation of let-7b in ovarian follicles may result in deregulation of the TGF-β signaling pathway and subsequently make contributions to PCOS development (47). Scalici et al. (2016) confirmed that in PCOS, estrogen receptor α expression changes which may impact miR-140 expression in ovarian follicles (47) contrarily. In PCOS ovaries, granulosa cells have more excellent proliferation rates associated with ovarian dysfunction (48). Jiang et al. (2015) demonstrated that miR-93 is excessively expressed in PCOS ovaries involved in promoting granulosa cell proliferation (49). Genes involved in IR and hyperandrogenism are getting altered by miRNAs from the principal contributing aspect of PCOS development.

An accelerated expression of various genes coding for steroidogenic enzymes was seen in ovaries of PCOS patients, which subsequently increases the production of the androgen (50). Different candidate genes engaged with androgen biosynthesis, transport, activity, and their regulation, in PCOS and functional hyperandrogenism are CYP17; steroidogenic factor 1: SF1; nuclear receptor subfamily: DAX-1; steroidogenic acute regulatory protein: STAR; CYP11A1, CYP11B2, CYP21, 3-beta-hydroxysteroid dehydrogenase: HSD3B2; HSD17B, CYP19, androgen receptor: AR; Estrogen receptor: ESR1; LHß; FSHß; FSH receptor; GnRH receptor; dopamine receptor; SHBG; glucocorticoid receptor and UDP-glucuronyltransferase 2B15 (51). Numerous miRNAs in human follicular liquids directs specific gene associated with steroidogenesis (Table 1) and play an essential function in PCOS progression (46). A key steroid biosynthetic enzyme, CYP11A1, is an expected target of miR-24-3p, and diminished levels of miR-24-3p may consequently lead to an enhanced expression of CYP11A1 and hyperandrogenism (52). A miR-29a-3p low level may intensify the hormonal imbalance deduced in PCOS by targeting STARD3 and AR, two anticipated target

genes of miR-29a-3p (52). ESR1 targets various miRNAs, including miR-193b, miR-222, and miR-520c-3p, among the other target genes, and others play out an absolute position in reproductive steroidogenesis approaches (46). Besides, Sang et al. (2013) saw miR-132, miRNA-24, miR-520c-3p, miRNA-222, and miR-320 controlled estradiol secretion. The mimic of miR-24 lowered estradiol secretion, and its inhibitor sped up estradiol secretion. miR-132, miR-320, miR-520c-3p, and miR-222 mimics increased estradiol secretion, and their inhibitors diminished estradiol secretion, and other miRNAs did not alter estradiol concentration (46).

At the cellular level, IR resulting from dampening phosphorylation of the insulin receptor substrate (IRS-1/IRS-2) in response to insulin tyrosine stimulation (53) and related downstream signalling exercises (translocation of glucose transporters to the cell membrane) in glucose-processing cells (54). Stress and inflammatory signaling activities (JNK activation) result in serine phosphorylation (instead of tyrosine) of insulin receptor proteins, inhibiting insulin signaling (cellular IR) (55). miRNAs-regulated genes are engaged with metabolic processes, such as insulin synthesis, secretion, sensitivity, and pancreatic ß-cells differentiation (Table 1) (56). For example, the overexpression of miR-29 prompted insulin obstruction in 3T3-L1 adipocytes (57), whereas miR-320 sped up insulin sensitivity (58), miR-30d inhibited insulin expression (59). Yuan and Tan (2017) recommended that miR-320 inhibits IR in patients with PCOS via regulating the ERK1/2 signaling pathway (60). Regardless of miR-143 being a positive controller of adipogenesis, miR-143-145 group knockout mice have been blanketed from overweight prompted IR, while restrictive overexpression of miR-143 results in exacerbated IR in diet-induced obesity. MiR-143 may also intensify IR by enhancing the degeneration of oxysterol-binding protein-related protein (ORP), a positive controller of Akt signaling (61). Experiments on transgenic mice have demonstrated that let-7 is a robust controller of glucose metabolism and peripheral IR by means of targeting IRS-2, insulin receptor (INSR), and insulin-like growth factor 1 receptor (IGF1R) in the liver and skeletal muscle (62). Shi et al. (2013b) revealed that overexpression of miR-181b slows down the signalling pathways PI3K/AKT and MAPK/ERK1, which is involved in IR (63). A study by Wu et al. (2014) shows that enhanced expression of miR-93 as observed in PCOS subjects' adipose tissue is positively correlated with IR (64). Subsequently, Chen et al., (2013) study unveiled that miR-93 overexpression outcomes in glucose transporter type 4 (GLUT4) downregulation in adipocytes via GLUT4 3'-UTR direct targeting, whilst inhibition of mir-93 activity led to sped up GLUT4 expression, showing GLUT4 dysregulation performs a central position in IR and PCOS (65). Furthermore, studies have demonstrated that miR-27b, miR-21, miR-155, and miR-103 are associated with regulating follicular development, androgen synthesis, and insulin sensitivity-related signaling pathways, which may additionally furnish a new target for the diagnosis and therapy of PCOS (60,66)

Epigenetic revamping of the biological clock in PCOS pathogenesis

Modulation of the circadian Clock gene led to the progression of diabetes, obesity, and metabolic disorders (67). Some studies showed the involvement of circadian genes in the pathogenesis of PCOS (68). The Clock mutant mice show hyperglycaemia, overweight and metabolic disorders (69). The Per1/Per2 double knockout mice and Cry1/Cry2 double knockout mice were intolerant to glucose (70,71). Mice without Bmal1 displayed similar intolerance to glucose and altered gluconeogenesis (72). Additionally, the standard circadian rhythm of elevated insulin sensitivity was completely nullified in global Bmal1 knockout mice (73). Muscle-specific BMAL1 inactivation and muscle CLOCK's ensuing disturbance cause a condition of diminished metabolic adaptability, described by muscle IR and adjusted glucose metabolism (7). It has been reported that siRNA-mediated inhibition of the clock gene interferes with the expression of aromatase (p450 arom) mRNA and, consequently, estrogen production (74). Genetic variant in CLOCK single-nucleotide polymorphism (SNP) rs1801260 (3111T/C) is connected with anomalies of lipid and glucose metabolism in the premenopausal female with PCOS, that underpins a capacity for the circadian clock in the progression of the metabolic disorder (67). Furthermore, hyperandrogenism, a frequent characteristic of PCOS, has been observed to alter the timing of clock gene expression in the liver and ovarian follicle in rats; both tissues are related to metabolic and reproductive function, respectively (12). Gonadotropin-releasing hormone (GnRH) neurons also express clock genes (75), which may be contributed to rhythmically via kisspeptin (76). The kisspeptin gene and its receptor have been linked with PCOS, and it is a potential target of endocrine and metabolic syndrome (77). Administration or deprivation of melatonin, a well-known marker of the circadian system, showed changes in the CYP17A1 expression and steroidogenesis in the ovary (78). The robust affiliation between SNP rs10830963 in the melatonin receptor (MTNR)1B gene (79) and SNP rs2119882 in the MTNR1A gene (80) and fasting glucose and insulin release and homeostatic model assessment for insulin resistance (HOMA-IR) point out that SNP, rs10830963 and SNP rs2119882 may be causative to the PCOS.

The circadian biology of the ovary

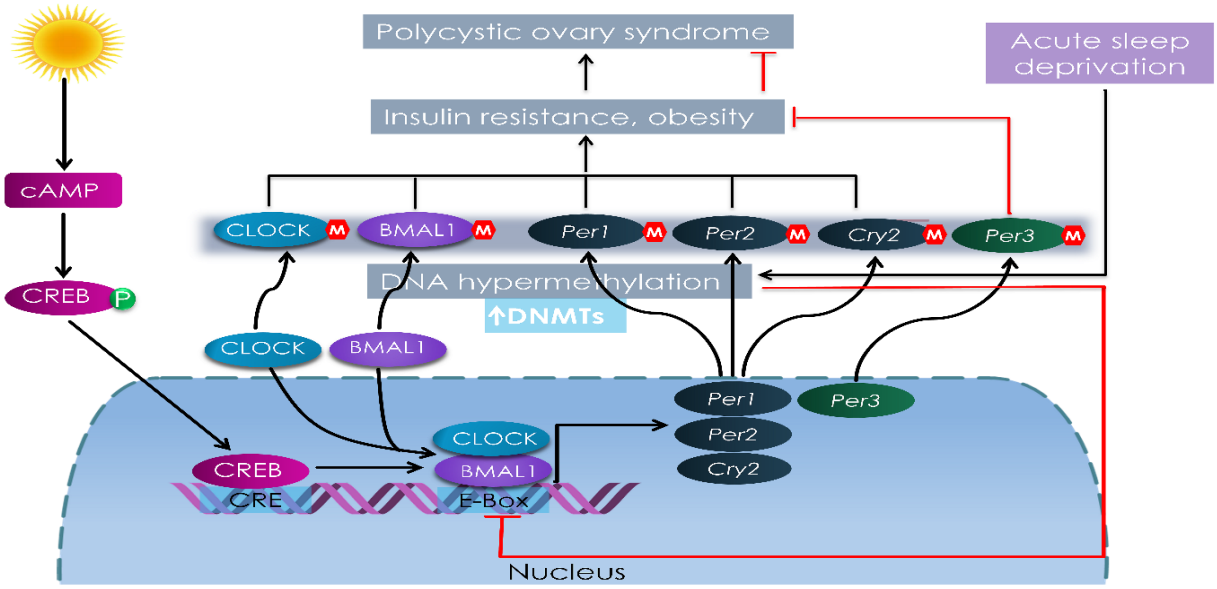

Figure 1. Schematic representation of circadian genes involved in the development of PCOS. Daylight favors the binding of CREB to the CRE situated within the target sequence. This coupling leads to CLOCK and BMAL1 heterodimer translocation into the nucleus and their interaction to the E-box promoter site of the target gene (*Per1, Per2, Per3, Cry1,* and *Cry2*). Enhanced expression of DNMTs causes hypermethylation of circadian genes involved in IR, and obesity adds to PCOS advancement. Hypermethylation of *Per3* is contrarily connected with IR and weight gain. Intense lack of sleep causes Hypermethylation of the circadian genes, which intern diminishes their expression.

The suprachiasmatic nucleus (SCN) in the hypothalamus regulates the mammalian clock (81,82). The circadian rhythm is a molecular clock comprising the transcriptional/translational feedback loops that manage the small variety of clock genes expression, which includes circadian locomotor output cycles kaput (Clock), brain and muscle ARNT-like protein1 (bmal1), period1 (per1) andcryptochrome1 (cry1) (48,83). PER and CRY proteins heterodimerized after they get translated to the cytoplasm and then phosphorylated with casein kinases (CK1). The phosphorylated PER and CRY complexes get translocated into the nucleus and suppress the CLOCK: BMAL1 complex. The expression of the clock gene in the ovaries might play a key role in ovulation. Prostanoid signalling is suggested to be critical in setting the circadian clock in the ovary and the time of ovulation (84,85). The transcription of COX2 is partly regulated via E-box DNA binding sequences that are recognized targets of the CLOCK: BMAL1 transactivator complex, therefore CLOCK: BMAL1 heterodimer may bind to and prompt COX2 transcription (86,87). The increased COX2 expression is also connected with the surge of luteinizing hormone (LH) via augmented prostaglandin synthesis, which may enable follicular rupture. Besides this, it has been pointed out that the circadian clock regulates steroidogenesis (88,89). In ovariectomized rats, LH and follicle-stimulating hormone (FSH) triggered the expression of PER1 and PER2 (90). The follicular cells in the ovary express receptors for melatonin and modulate the ovarian clock. The ovulation timing not only depends on the SCN dependent secretion of LH but also gonadotropins timing which is set through the ovarian clock (91).

DNA Methylation

The transcripts for the centre oscillator factors such as ARNTL, CLOCK, PER1, PER2, and CRY1 have existed in the ovary of rodents (92). Herein, we describe the fundamental role of circadian genes in the advancement of PCOS. Studies have demonstrated the lower BMAL1 expression in the granulosa cells of women with PCOS. The aromatase expression and estrogen synthesis were downregulated in KGN cells following BMAL1 knockdown while upregulated in cells overexpressing BMAL1 (68). Blockade of CREB signaling showed a colossal decrease in arrhythmic phenotype and light-evoked clock entrainment (93). CREB methylation levels positively correlated with body mass index (BMI) (94). The long-term shift worker displays weight problems and metabolic syndrome risk, which may be associated with the hypomethylation of the Clock gene promoter and Cry2 hypermethylation in peripheral blood DNA (18). Further, sleep disturbances in the form of obstructive sleep apnoea and daytime sleepiness have been seen in females with PCOS (95). Cedernaes et al. (2015) also noted that lack of sleep resulted in the augmentation of promoter methylation and diminished translation of circadian genes in several tissues. They showed expanded methylation of transcription-regulating areas of Per1

and Cry1 in adipose tissue and decreased gene expression of Cry1 and Bmal1 in skeletal muscle. Centre clock gene hypermethylation is connected to IR in people **(Figure 1**) (19), which is one of the essential contributing factors for PCOS progression (96).

boxes and prompts its transcription (**Figure 2**). The circadian difference in insulin sensitivity involved CLOCK/BMAL1 and Sirt1. Circadian disruption decreases the hepatic BMAL1 and Sirt1 and induces IR (21). Zhang et al. (2016) also showed the consequences of BMAL1 on oestrogen synthesis in

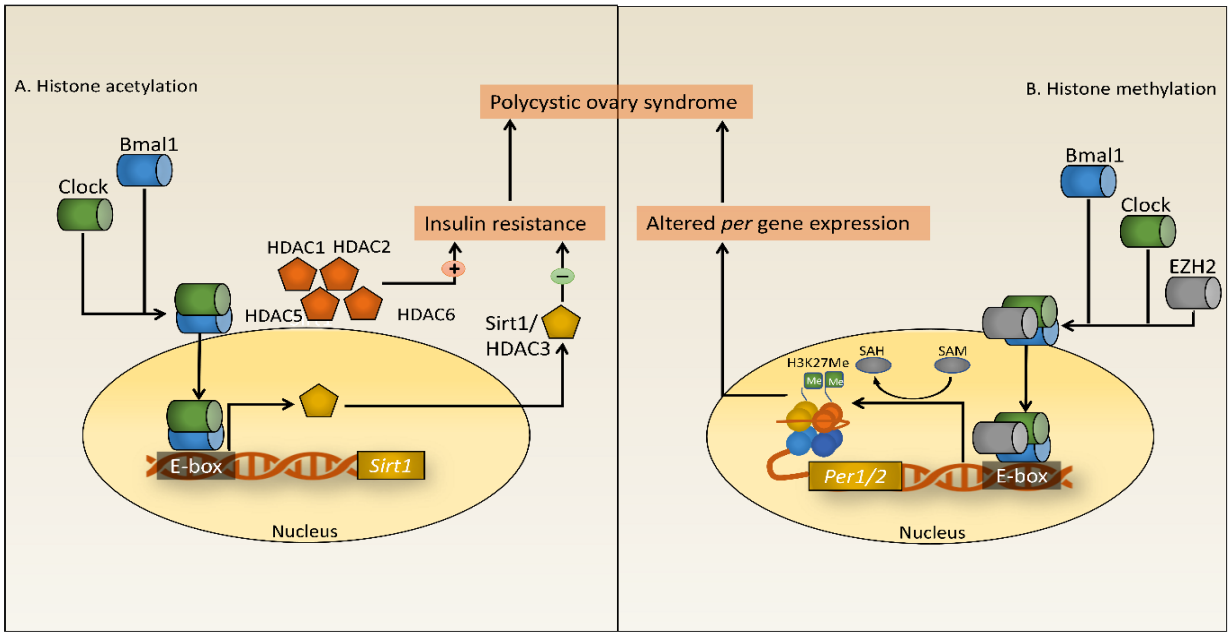

Figure 2. Histone modifications in circadian genes in the progression of PCOS. A: Histone acetylation: CLOCK/BMAL1 heterodimer binds to the Sirt1 (HDAC3) promoter region containing E-box and activates its transcription. Resultant Sirt1 reduces IR. Other HDACs, including HDAC1, HDAC2, HDAC5, and HDAC6, promote IR resulting in PCOS; B: Histone methylation: The histone methyltransferase, EZH2, interacts with CLOCK–BMAL1 complexes and is recruited to the Per1/2 promoters, where it catalyzes the methylation of histone H3 at lysine 27 (H3K27), resulting in transcriptional repression and per gene expression alteration which contributes to PCOS progression.

Histone modification

A focal component of the circadian pacemaker CLOCK protein is taking action like HAT, which causes acetylation of histone proteins and non-histone proteins (20). Active enhancer markers like H3K27Ac and H3K9Ac have been demonstrated to be rhythmic and related to clock gene expression (97). At the Clock loci, rhythmic histone acetylation commonly intervened by means of p300 and CBP HATs (98). Overexpression of HDAC5 in mouse fibroblasts affects the transcriptional rhythms of clock genes. HDAC5 overexpression on the Lys-537 diminishes BMAL1 acetylation, and pharmacological modulation of HDACs of class IIa expands BMAL1 acetylation (99). The sirtuins (Sirt1), a unique class III HDACs, have displayed extensive investigational momentum (100). Sirt1 manages the circadian epigenetic via H3K9 deacetylation, acetylation of Bmal1 and Per2 (101–103). The role of Sirt1 is well proven in body weight, cardiovascular problems, and diabetes (104–106). The importance of Sirt1 has also been demonstrated in the immune system, inflammation, and insulin metabolism (107). Zhou et al. (2014) unveil that CLOCK/BMAL1 binds to the Sirt1 promoter vicinity containing the two E-

human chorionic gonadotropin (HGC). They suggested the importance of BMAL1-SIRT1-JNK feedback mechanisms in the PCOS progression (68). The process of histone methylation is important in the regulation of the circadian clock (Figure 2). Interaction occurs between zeste homolog 2 (EZH2) a histone methyltransferase, CLOCK and BMAL1 and complex forms which subsequently recruited to the promoters of Per1 and Per2, where it catalyses the H3K27 methylation, generally considered as a sign of transcriptional suppression [95]. Ma et al., (2017) demonstrated that androgens acts via PI3K/Akt pathway phosphorylate and repress the action of EZH2, which recommends the connection between histone methylation, circadian clock and PCOS (108).

Circadian miRNA

miRNAs may play an irreplaceable role in modulating the circadian clock (**Figure 3**). The dysregulated miRNA expression showed a remarkable change in the circadian timing and output (**Table 1**) (109). miR-219 and miR-132 are the two brain explicit miRNAs that majorly impacted the

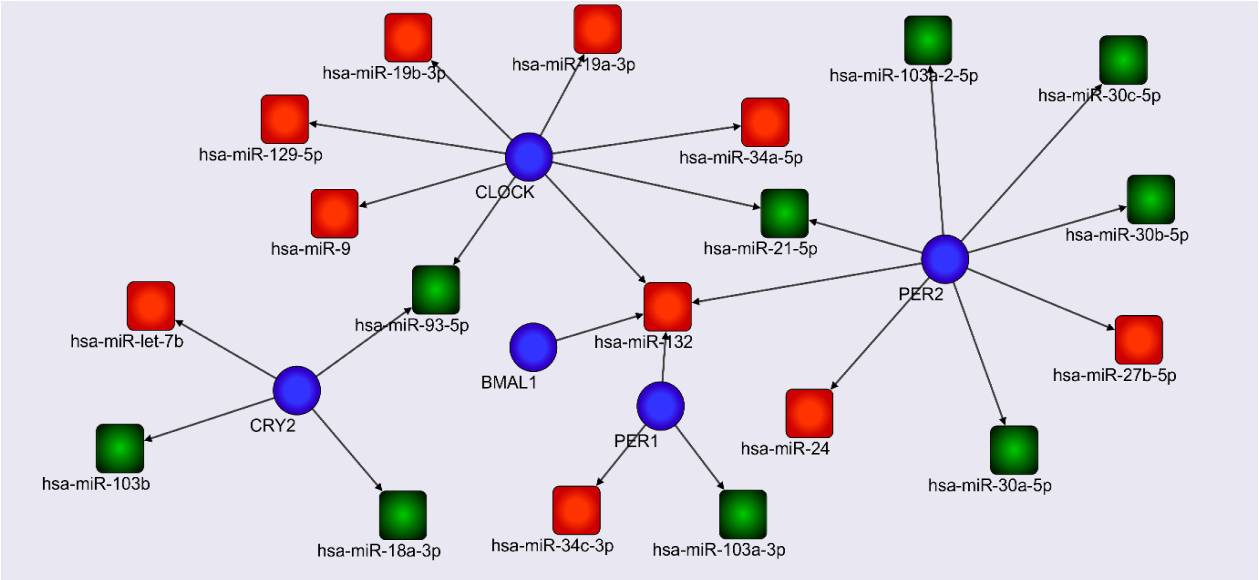

Figure 3. miRNA networking of circadian gene and miRNAs implicated in PCOS. Green: upregulated miRNAs; Red: downregulated miRNAs; Blue: circadian gene. Network plotted to utilize interaction table from mirwalk 2.0 and visualized by Cytoscape v3.6.0

SCN core located circadian clock (110). Overexpression of miR-132 showed an expanded CLOCK/BMAL, and K+/forskolin instigated mper1 transactivation (111). The miR-132 transgenic mice showed the diminished light-prompted resetting of behavioral rhythms (112). Sang et al. (2013) study unveiled that miRNA-132 is substantially related to PCOS. Moreover, diminished levels of miR-132 were noticed in gestational diabetes mellitus, suggesting miR-132 may regulate insulin secretion (46,112). Similarly, the involvement of the miR-93 was reported in the circadian mechanism by targeting Clock and Cry2 genes (113). In PCOS-affected women, increased miR-93 expression (65). miR-24-3p was observed to regulate a key steroid biosynthetic enzyme, CYP11A1. The diminished levels of miR-24-3p might consequently lead to an elevated CYP11A1 expression that ultimately results in hyperandrogenism and PCOS. A forkhead transcription aspect encoded by FOXL2 is essential for the development of the ovary (114). In cultured human granulosa cells, overexpression of miR-30a promotes expression of immediate early response 3 (IER3), cyclin D2, and BCL2A1, by means of forkhead Box L2 (FOXL-2) suppression (59). Conditional knockout of FOXL-2 showed an elevated androgen production by theca cells and granulosa cells and morphological transformation such as in PCOS (47,115). The reduced expression of miRNAs 146a and 146b and elevated expression of activated NF-κb correlated with the circadian disruption (116). The miR-146a serum level is negatively related to testosterone level, which may serve as a novel biomarker for PCOS analysis (117). Let-7b is also involved in controlling the circadian oscillation and is predicted to target cry2 (116). Furthermore, it has been proven that down-regulation of let-7b in the follicles in the ovary could lead to deregulation of the TGF-β signaling pathway that further leads to susceptibility to PCOS (47). The Per3a circadian gene is also proposed as a novel target of miR-103 (118). Additionally, the expression of miR-103 in granulosa cells reduces obesity and PCOS.

Conclusion and future perspectives

The disturbed epigenetic programming has been demonstrated in several human diseases (14,124). The changes in core circadian genes are associated with the development of obesity, diabetes, and metabolic syndrome in mice (67). Recently, the importance of circadian genes in the development of PCOS is growing (68). Several studies have stated that the epigenetic modifications of circadian genes in the progression of IR and hyperandrogenism, which is also contributed to PCOS. Scientific evidence suggests that circadian-related epigenetic changes triggered by circadian miRNAs and other epigenetic modifiers are highly correlated with IR, hyperandrogenism, and PCOS pathogenesis. Thus, the importance of circadian epigenetics is becoming highly relevant. A growing body of evidence proposed several epigenetics-based mechanisms in the pathogenesis of PCOS. For instance, the DNA methylation, histone modifications, and miRNA-related alterations of circadian genes are well reported in the PCOS. This review has also generated few questions as below i) do DNA methylation, histone modifications, and miRNA-related alterations in circadian genes together find a common underlying mechanism for the development of PCOS; ii) what is the possibility of crosstalk between circadian miRNAs and the circadian gene epigenetic modifiers for the etiopathogenesis of the PCOS; and iii) what is the

Table 1. Role of miRNAs in PCOS

MiRNA	Location	Target gene	Expression	Function	Reference
miRNAs regulating steroidogenesis					
miR-24-3p	Follicular fluid Blastocyst	*CYP11A1*	Down	Decreases androgenism, decreases TGF signalling decreases estradiol secretion	(119)
miR-29a-3p	Follicular fluid	*STARD3, AR, PTEN*	Down	Decreased steroid production, Increases cell growth via inhibition of PTEN	(52)
miR-132, miR-320, miR-222, miR-520c-3p	Follicular fluid	*ESR1*	Down	Increases estradiol secretion	(46)
miR-24	Follicular fluid	*ESR1*	Up	Decreases estradiol secretion	(46)
miRNAs regulating insulin resistance					
miR-29	Adipocytes		Up	IR	(57)
miR-320		*ERK1/2*	Down	IR via altering the ERK1/2 signaling pathway	(58,60)
miR-30d			Up	Inhibits insulin expression	(59)
miR-143		*Akt*	Up	IR in diet-induced obesity via affecting Akt signaling	(61)
Let-7	Skeletal muscles, Liver tissue	*IRS-2*	Down	Regulator of glucose metabolism and peripheral IR	(62)
miR-181b		*Akt/MAPK*	Up	IR, slow down PI3K/Akt and MAPK/ERK 1 pathway	(63)
miR-93	Adipocytes	*GLUT4*	Up	Downregulates GLUT4 and thereby IR	(64,65)
miR-21, miR-27b, miR-103, and miR-155				Involved in the regulation of follicular development, androgen synthesis, and insulin sensitivity-related signaling pathways	(60,66).
miRNAs regulating circadian gene					
miR-132	Follicular fluid	*Clock /bmal1 Per1* and *Per2*	Down	Enhance insulin secretion (also in T2DM)	(110–112)
miR-93	Adipose tissue	*Clock* and*cry2*	Up	IR	(113)
miR-24		*Per2, CYP11A1*	Down	Increased expression of CYP11A1 and thereby hyperandrogenism	(120,121)
miR-30a	Granulosa cells	*Per2 FOXL-2*	Up	RepressingFOXL-2 results in cystic follicles.	(59,121)
miR-146a		*Per1*	Down	Increased testosterone level	(116,117)
Let-7b	Ovarian follicle	*Cry2 TGF-β*	Down	TGF-β signaling pathway deregulation	(47,116)
miR-103	Granulosa cells	*Per3*	Up	Promotes progesterone release while inhibiting estradiol release and also elevate testosterone level	(118,122,123)
miR-132	Follicular fluid	*Clock /bmal1 Per1* and *Per2*	Down	Enhance insulin secretion (also in T2DM)	(110–112)
miR-93	Adipose tissue	*Clock* and*cry2*	Up	IR	(113)
miR-24		*Per2, CYP11A1*	Down	Increased expression of CYP11A1 and thereby hyperandrogenism	(120,121)

key role of post-transcriptional changes such as phosphorylation, ubiquitination, sumoylation and ADP-ribosylation in histone tails in the progression of PCOS. We believe that epigenetic alteration plays an indispensable role in modulating the circadian clock, which may be a critical step in the pathogenesis of PCOS (109).

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Conflict of interest

The authors declare no potential conflicts of interest

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