Reproductive Sciences (2022) 29:680–694
https://doi.org/10.1007/s43032-021-00516-3

REVIEW

Polycystic Ovary Syndrome: the Epigenetics Behind the Disease

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Received: 2 September 2020 / Accepted: 21 February 2021 / Published online: 7 April 2021
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Abstract

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting approximately 5–20% of women of reproductive age. PCOS is a multifactorial, complex, and heterogeneous disease, characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries, which may lead to impaired fertility. Besides the reproductive outcomes, multiple comorbidities, such as metabolic disturbances, insulin resistance, obesity, diabetes, and cardiovascular disease, are associated with PCOS. In addition to the clear genetic basis, epigenetic alterations may also play a central role in PCOS outcomes, as environmental and hormonal alterations directly affect clinical manifestations and PCOS development. Here, we highlighted the epigenetic modifications in the multiplicity of clinical manifestations, as well as environmental epigenetic disruptors, as intrauterine hormonal and metabolic alterations affecting embryo development and the adulthood lifestyle, which may contribute to PCOS development. Additionally, we also discussed the new approaches for future studies and potential epigenetic biomarkers for the treatment of associated comorbidities and improvement in quality of life of women with PCOS.

Keywords Polycystic ovary syndrome · Metabolic syndrome · Epigenetics · DNA methylation · ncRNA · Histone modifications

History and Pathophysiology of PCOS

The first report of polycystic ovary syndrome (PCOS) was noted more than 2000 years ago when Hippocrates (460 AC-377 AC) related menstrual disorders in "robust" healthy women with "masculine characteristics" [1]. In 1721, Vallisneri described a case of an infertile woman with bright white surface ovaries of the size of pigeon eggs [2]; in the following years, many reports of women with symptoms similar to PCOS emerged, but it was not then systematized. In 1935, Stein and Leventhal described for the first time the most similar phenotype related to PCOS, which included women with menstrual disorder, hirsutism, and enlarged ovaries with small follicles, later referred to as the Stein–Leventhal syndrome [3, 4].

Despite the advances in the understanding of the pathophysiology and treatment of PCOS-related symptoms, the diagnosis criteria were first established in a conference at the National Institutes of Health (NIH) in 1990. PCOS was classified as chronic anovulation or ovulatory dysfunction due to excessive presence of androgens, in the absence of any other disorders with similar symptoms [5]. In 2004, this classification was revised in the Rotterdam consensus, with inclusion of the size and morphology of the ovaries in the diagnosis. According to this classification system, PCOS must present at least two of the following three characteristics: (1) hyperandrogenism (clinical and/or biochemical), (2) ovulatory dysfunction, and (3) polycystic ovaries, with 12 or more cysts in one ovary and/or ovarian volume of >10 mL [6].

The Rotterdam criteria are well accepted and widely used, although in 2006 the Androgen Excess Society issued a statement in an attempt to establish hyperandrogenism as a key condition for PCOS diagnosis, excluding cases in which only

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ovulatory dysfunction and polycystic ovaries are the main features underlying the pathophysiology of chronic anovulation and infertility observed in PCOS women [7]. Insulin resistance (IR) [8] and metabolic alterations, such as obesity, dyslipidemia, arterial hypertension, abnormal glucose metabolism, and chronic inflammation are common disorders associated with the pathophysiology of PCOS, which increases the risk of developing *type 2 diabetes mellitus* (T2DM) and cardiovascular disease (CVD) [9]. The prevalence of PCOS may vary depending on the diagnosis criteria; it affects 5–20% of women worldwide, thereby being the main cause of anovulatory infertility [10]. The large variation in the number of affected women reflects the heterogeneous nature of PCOS phenotypes and associated comorbidities, making it difficult to understand the etiology of this syndrome [11]. This suggests a complex and multifactorial origin of PCOS, in which genetic alterations, environmental factors, and epigenetic modifications contribute to its development or the worsening of its clinical manifestations [12].

Genetic Basis of PCOS

The large number of clinical phenotypes is mainly due to hormonal and metabolic disturbances that directly affect the reproductive outcomes in women with PCOS. Familial studies strongly support the genetic origin of PCOS; most of them suggest autosomal dominant inheritance with incomplete penetrance and variable expressivity due to a combination of multiple genetic components and environmental interactions [13]. The genetic origin of PCOS is confirmed by a hereditary factor observed in first-degree relatives, since mothers or sisters with PCOS are at an increased risk (35–40%) of developing the disease [14]. In addition, the susceptibility of the disease in monozygotic twins is at least twice as high as in dizygotic twins [15].

Despite the clear genetic influence, it is unlikely that a single gene is responsible for the onset of the disease. Genome-wide association studies (GWAS) have identified a large number of variants involved in crucial pathways related to endocrine disturbances and hyperandrogenism observed in PCOS pathophysiology [16–20]. A intricate network involved in the regulation of cell proliferation, insulin metabolism, follicular growth and maturation, hormonal regulation, and diabetes susceptibility are required for ovarian steroidogenesis and oocyte maturation and PCOS development [21–40]. Single nucleotide polymorphisms (SNPs) and repetitive DNA sequences have been associated with several aspects and increased risk of PCOS development. Among the main molecular markers reported in GWAS, most are involved in androgen biosynthesis and metabolism, insulin resistance, oxidative stress, and T2DM. The genetic variants were mainly located in the *LHCGR*, *FSHR, CYP11A*, *THADA*, *ERBB4*, *GATA4*, *HSD17B5*, *FSHB*, *HMGA2*, *RAB5B/SUOX*, *INSR*, and *H6PD* genes that play an important role in PCOS susceptibility (Table 1). Genes involved in cellular growth and apoptosis are related to the reproductive outcomes of PCOS, including ovarian dysfunction, folliculogenesis, and infertility (Table 1).

Repetitive DNA sequences are related to transcriptional regulation of gene expression and the maintenance of genomic stability [41]. Triplet repeat sequences are associated with several human diseases, including reproductive disorders. The repetitive (CAG)n polymorphism in the androgen receptor gene (*AR*) has a physiological role in ovarian function and folliculogenesis and may also be a risk factor for PCOS development. The increased number of CAG repeats negatively correlates with *AR* activity, which affects androgen action in women with PCOS [42]. Alterations in the repetitive telomeric region are also related to PCOS; however, the results are contradictory, as they are directly affected by clinical characteristics [43–45]. Metabolic disturbances observed in PCOS may contribute to the progressive telomeric loss, leading to cellular senescence, reproductive aging, and chronic anovulation. However, the excess androgen may stimulate telomerase activity and protect against telomere erosion [46].

Although the genetic origin of PCOS is widely supported, the underlying molecular mechanisms and pathways remain unexplored. The genetic contribution to PCOS pathophysiology involves a complex network of interactions; various genes and pathways are associated with these interactions. However, the common variants reported have a small effect on disease development and do not explain the variety of clinical alterations observed in PCOS [47]. The identification of rare genetic variants using high-throughput genetic strategies and the regulation of gene expression may contribute to a better understanding of PCOS pathophysiology. Indeed, environmental changes during the lifetime, whether intrauterine or after birth through adulthood, contribute to the heterogenic phenotype of PCOS, suggesting an epigenetic component as the main cause of its development, as well as the associated endocrine and hormonal disturbances.

Epigenetic Fetal Origin of PCOS

Epigenetic marks, such as DNA methylation, histone modifications, and noncoding RNA regulation, modify the structure of chromatin, conferring a differential program of gene expression, irrespective of changes in the DNA sequence [48]. Epigenetic modifications control gene expression dynamically and reversibly, as the epigenome of a cell has great plasticity and can be reprogrammed. Epigenetic reprogramming changes cell fate throughout development and adulthood, and environmental factors play a crucial role in the maintenance and establishment of epigenetic marks [49]. Disruptions in the epigenetic machinery are related to the etiology of various neurodevelopmental disorders, such as the Beckwith–

Table 1 Main candidate genes for PCOS

Gene	Description	Possible relation with PCOS	Reference
LHCGR	Encodes LH and hCG receptors	SNPs associated with PCOS risk	[16, 19, 20, 37]
FSHR	Encodes FSH receptor	Stimulates oogenesis, follicular development, gametogenesis, follicular maturation, and proliferation of granulosa cells and PCOS risk	[16, 20, 23]
FSHB	Encodes the hormone-specific β-subunit of FSH	Related to ovarian follicle growth, estrogen production and SNP related to PCOS risk	[16, 24]
THADA	Encodes thyroid-adenoma associated protein	SNPs related to type 2 diabetes and PCOS risk	[16, 24, 25]
ERBB4	Encodes a member of *EGFR* family	Variants related to PCOS and late follicular maturation	[24]
GATA4	Encodes a zinc-finger transcription factor	Regulate gonadal development, the transcription of steroidogenic genes and locus associated with PCOS	[16]
HSD17B5	Related to testosterone-forming in females	SNP and polymorphism related to increased risk of PCOS and testosterone excess	[28, 29]
HMGA2	Encodes a protein member of the non-histone chromosomal *HMG* family	Nearby SNP related to PCOS risk	[23]
RAB5B/SUOX	Encodes a member of the *RA5* family	Nearby SNP related to PCOS risk	[16, 23]
CYP11A1	Related to sex hormones synthesis	Repetition polymorphism related to PCOS risk	[31]
INSR	Encodes the insulin receptor gene	SNPs related to PCOS risk and insulin metabolism	[23, 32, 34, 35]
H6PD	Related to oxo-reductase activity	Polymorphisms related to PCOS risk and hyperandrogenism	[36]
HSD11B1	Encodes 11-beta-hydroxysteroid dehydrogenase type 1 and adjust the oxo-reductase activity	Polymorphisms related to PCOS risk and hyperandrogenism	[36]
YAP1	Related to regulatory organ volume pathways and ovarian cancers	SNPs related to PCOS risk	[16, 38]
DENND1A	Encodes a member of the connecdenn family	Related to higher PCOS risk via altered activity of endoplasmic reticulum aminopeptidase 1	[16, 25]
HSD17B6	Androgenic metabolism	SNPs related to PCOS hyperandrogenism	[29]
RAD50	Involved in DNA double-strand break repair	Polymorphisms related to PCOS risk	[24]
KRR1	Involved in nucleolar processing and ribosome assembly	Polymorphisms related to PCOS risk	[24]
IRS2	Regulate the development of ovarian follicles and act as an antagonist to aromatase activity	SNPs related to insulin metabolism and PCOS risk	[35]
FST	Encodes follistatin protein	SNPs related to androgenic phenotypes of PCOS	[39]
FBN3	Encodes de fibrillin 3 protein	Variants related to PCOS reproductive and cardiovascular abnormalities	[40]

Wiedemann, Silver–Russell, and Fragile X syndrome [50, 51], as well as complex and multifactorial diseases such as cancer [52], T2DM, and metabolic alterations [53].

Owing to the multifactorial and complex etiology of PCOS, epigenetic disturbances may be the main alteration underlying the development and severity of this disorder. Environmentally induced epigenetic changes caused by an intrauterine or postnatal adverse environment may trigger PCOS-like symptoms or its associated clinical alterations after birth [54, 55]. Metabolic and hormonal dysfunctions during fetal development may promote aberrant epigenetic reprogramming, leading to physiological changes in the fetus and PCOS emergence in adult life. This hypothesis may also explain the familial genetic basis and susceptibility to acquiring PCOS in first-degree relatives [56].

Hyperandrogenism seems to be the main factor underlying the fetal origin of PCOS, as the intrauterine androgen excess appears to predispose to PCOS in adulthood via epigenetic mutations. This has been observed in various studies, wherein the embryo exposed to the hyperandrogenic environment during prenatal life presented PCOS-like alterations, such as elevated levels of testosterone, progesterone, estradiol, and luteinizing hormone (LH), irregular estrous cycles, reduced preovulatory follicles, and increased preantral follicles [57–60]. In addition, Rhesus monkeys exposed to high levels of androgens during fetal development reportedly presented a PCOS-like phenotype after birth [61].

Altered DNA methylation is the main reported epigenetic marker in developmental epigenetic reprogramming of PCOS. These modifications lead to gene silencing or reduction of its activity and regulation of important developmental processes, including genomic imprinting [62] and X-chromosome inactivation [63], as well as the transcriptional and posttranscriptional regulation of gene expression and chromatin remodeling [64]. In a previous study, the hyperandrogenic intrauterine environment altered the DNA methylation patterns of prenatally androgenized Rhesus monkeys, with 163 genes in infants and 325 in adults, especially those linked to

the antiproliferative transcription factor TOB in T cells and the transforming growth factor-ß (TGF-β) signaling pathways, such as *TGFBR1*, *KRAS*, *BMP2*, *TFE3*, *RUNX3*, and *HOXC8* [61]. Previous studies have also implicated members of the TGF-ß superfamily, such as fibrillin-3 and inhibin B, in the pathophysiology of PCOS [40].

Androgen excess during prenatal life lead to DNA hypomethylation in androgenized rats at specific CpG sites from the promoter region of *GATA6* (-520) and *STAR* (-822) genes involved in steroidogenesis and steroid biosynthesis [65]. In addition to the influence of androgens, other external factors may alter the epigenetic landscape, thus leading to PCOS. Rats exposed to the agricultural fungicide vinclozolin and the insecticide dichloro-diphenyl-trichloroethane (DDT), reportedly presented changes in the transcriptome and epigenome of ovarian granulosa cells in the F3 generation, suggesting an epigenetic transgenerational inheritance of ovarian pathology [66].

Assuming that hyperandrogenism during development play a role in the emergence of PCOS in adulthood, the information regarding the time of development and the endocrine disruptors that can lead to PCOS phenotype and/or ovary dysfunction, is crucial for a better understanding of the mechanisms underlying this syndrome. One hypothesis is that the major influence of androgen levels occurs during mid-gestation when intrauterine hyperandrogenism can affect fetal development, including ovarian morphological alterations and sex steroid production [67–69]. Ewes prenatally treated with testosterone showed growth retardation, reduced ovarian reserve, and increased ovarian follicular recruitment [70]. However, the origin of PCOS due to excess androgen may occur earlier in the development process depending on the species. Zebrafish embryos exposed to androgens in early development presented changes in global DNA methylation levels in ovaries, but these alterations were not observed during late androgen exposure. Early embryonic androgen exposure seems to be transferred to offspring with transgenerational changes in the ovarian epigenome and glucose homeostasis [71].

Echiburú and colleagues (2020) analyzed the DNA methylation profile in 368 CpG sites in the promoter regions of seven reproductive and metabolic genes (*LEP*, *ADIPOQ*, *AMH*, *LEPR*, *ADIPOR1*, *ADIPOR2*, and *AR*) in children born to women with PCOS treated with metformin during pregnancy. Daughters of the PCOS women showed differences in one CpG site of *LEPR* (Chr1-65419664 site, hypermethylation) and *ADIPOR2* (Chr12-1690290, hypermethylation) genes and two CpG sites in the *AR* gene (ChrX-67543969 and ChrX-67544981 sites, hypomethylated) when compared to the non-PCOS controls. In sons, five CpG sites in *LEP*, three in *AMH*, and nine in *AR* genes showed differences in methylation levels between the groups. Moreover, the Chr7-128240906 and Chr7-128241078 sites in *LEP* exhibited higher methylation levels in the infants from PCOS women treated with metformin. The authors suggested that an intrauterine PCOS environment predisposes a sex-dependent DNA methylation pattern [72].

Epidemiological studies support the adverse intrauterine environment linked to health problems in adult life as developing PCOS. Birth weight studies suggest that hyperandrogenism is more prevalent in infants small for gestational age, and the prevalence of PCOS is twice as high as in the infants appropriate for gestational age [73]. Increased levels of testosterone during prenatal life are related to other developmental disorders, as women with PCOS present autistic characteristics and a greater predisposition to having a child with autism [74]. Alterations in the androgen receptor (*AR*) gene and non-random (skewed) X chromosome inactivation (XCI) may play a role in PCOS development in early gestational age. The repeat trinucleotide sequence is differentially methylated in the active and inactive X-chromosomes, which is usually a random process [75]. Although random XCI seems to be present in PCOS, the skewed XCI is related to shorter CAG repeats in the *AR* gene [76–79]. In addition, the *AR* CAG repeats and the nonrandom XCI appear to exert considerable effects on LH and FSH levels [80].

Despite the growing evidence of developmental PCOS and aberrant epigenetic reprogramming during embryonic and fetal life, the related pathways and the mechanisms underlying the inherited PCOS remain unclear. However, the hyperandrogenic intrauterine environment and other endocrine disruptors induce reproductive and metabolic dysfunctions in adulthood via epigenetic mutations [55]. The relationship between hyperandrogenism and metabolic intrauterine exposure during development provides new insights regarding familial inheritance and the environmental influence on PCOS development and its related comorbidities; this information also contributes to the development of intrauterine preventive treatments in the offspring.

PCOS Comorbidities and Epigenetic Markers in Adulthood

The genome of an individual responds in a coordinated way to environmental changes during life [49]. Besides mutational events, disruption in the epigenetic control of gene function also alters many biological processes and is related to the pathophysiology of human diseases. The multifactorial nature of PCOS and the diversity of clinical features observed suggest that genetic predisposition and environmental changes alter the epigenome and may be the main alteration behind its etiology. Aberrant epigenetic reprogramming through endocrine disruptors or lifestyle changes may lead to PCOS development and play a role in ovarian alterations and reproductive dysfunctions related to this disorder [81]. The epigenetic biomarkers, as well as their modifications, diagnostic

potential, and tissues analyzed in PCOS, are summarized in Table 2.

The altered epigenetic biomarkers in PCOS seem to participate in an intricate network of biological interactions, as shown in Fig. 1. Based on the biomarkers suggested in Table 2, a functional network among PCOS-interacting proteins was predicted using the STRING database (https://string-db.org, version 11.0) [105]. The network interactions were built with a score of 0.4 or greater, using multiple proteins, selecting *Homo sapiens* as an organism and an initial input of 28 proteins; the scores obtained for each interaction are summarized in Supplementary Table S1. This network had significantly more interactions than expected, for a random set of proteins of similar size drawn from the human genome (PPI enrichment p-value: 2.42e−08). This enrichment indicates that the proteins are biologically connected. The five main functional enrichments in the PCOS-interacting protein network based on the highest false discovery rate are presented in Table 3. According to gene ontology (GO) analysis, these proteins participate in important biological processes and molecular functions related to response to chemical and organic stimuli, signaling pathways, and hormonal function, and regulate reproductive function metabolic disturbances pathways in PCOS.

Altered DNA methylation in genes related to androgen production, insulin resistance, and other metabolic alterations was reportedly observed in PCOS [106]. A genome-wide DNA methylation study of peripheral blood identified 52 differentially methylated CpG sites related to different functions such as significant functional pathways and important clinical characteristics of PCOS women, such as prolactin, estradiol, and progesterone levels, and menstrual cycle alterations [107]. Consecutive hypomethylated CpG sites have been identified in the promoter region of the *EPHX1* gene in the peripheral blood of PCOS [88]. Hypomethylation of the repetitive element LINE-1 (Long interspersed nuclear elements 1) in blood leukocytes is strongly associated with the susceptibility and hormonal changes in PCOS [83].

The identification of epigenetic changes related to endocrine and metabolic disturbances in PCOS, such as IR, hyperinsulinemia, obesity, and other related conditions, contributes to a better understanding of the mechanisms involved in the pathogenesis of this disease. Insulin resistance (IR) is one of the main comorbidities in women with PCOS. In peripheral blood, Shen and colleagues (2013) identified 79 genes differentially methylated between PCOS with insulin resistance (PCOS/IR) and without IR, of which 40 genes were differentially methylated between PCOS and non-PCOS women [108]. Another study also showed that the *LMNA* gene, related to metabolic alterations such as hyperandrogenism and insulin resistance, was hypermethylated in PCOS women with IR [93]. Changes in DNA methylation of specific genes and promoter regions are closely related to PCOS development [87, 91, 109, 110]; however, global DNA methylation patterns in PCOS are poorly understood and controversial. No difference in global DNA methylation has been reported in PCOS-women when compared to non-PCOS controls [81]. In contrast, Sagvekar et al. (2017) demonstrated a reduction of ~25% in global DNA methylation in PCOS women [83].

Epigenetic modifications seem to be different in obese and nonobese PCOS. Obesity or overweight is a common characteristic of PCOS women, which also affects the clinical manifestations, such as IR, hyperinsulinemia, and hyperandrogenism [111]. Decreased DNA methylation and overexpression of the *LHCGR* gene were observed in subcutaneous adipocytes of non-obese PCOS, while the *INSR* gene showed decreased DNA methylation and underexpression in those with obesity [96]. DNA methylation profile may be useful in identifying the risk of developing PCOS metabolic disorders. Hypermethylation of the *PPARGC1A* promoter was observed in leukocytes from PCOS women, while mitochondrial DNA content decreased. The higher the metabolic risk, the greater the difference observed [95].

Besides altered DNA methylation, disruption in non-conding RNAs (ncRNAs) expression is related to PCOS and its related comorbidities. ncRNAs are epigenetic marks that control gene expression at the transcriptional level by binding to DNA or RNA sequences, and at posttranscriptional level by binding to the protein. The main classes of ncRNAs are microRNAs (~20 nt) and long ncRNAs (>200 nt), which are related to several biological processes [112, 113]. In this context, miR-93 was identified as overexpressed in the adipose tissues of women with PCOS, PCOS/IR, or non-PCOS/IR compared to that in the non-PCOS/non-IR controls, while its target, the *MCM7* gene, was downregulated in PCOS and non-PCOS/IR, with the lowest expression levels being in the PCOS/IR [99]. A previous study reported the upregulation of miR-93, miR-133, and miR-223 in PCOS women; however, the predicted target for miR-93 was the *GLUT4* gene, an insulin-sensitive glucose transporter that is downregulated by miR-93 overexpression and may be responsible for PCOS/IR [100]. In addition, the downregulation of lncRNA *GAS5* and elevated expression of the *IL-18* gene in the serum of PCOS patients might contribute to IR [114].

In addition to their influence on IR, the miRNAs are also related to obesity, which is often described as a PCOS comorbidity. The levels of miR-21, miR-27b, and miR-103, associated with metabolic disorders such as obesity and diabetes, are downregulated in the whole blood of obese women without PCOS; however, in those with PCOS, these miRNAs are overexpressed [104]. In women with PCOS, serum expression of miR-222, miR-146a, and miR-30c increases significantly and miR-222 levels are positively associated with insulin levels, while miR-146a levels are negatively associated with testosterone levels [103]. In addition, Qin and colleagues (2019) demonstrated that high expression levels of lncRNA *H19* were associated with a high risk of PCOS, and its expression was positively correlated

Table 2 Epigenetic biomarkers and alterations involved in the PCOS

Cell type	Gene	Methylation status	Gene expression	Biomarker to	Reference
Granulosa cells	*LHCGR*	Hypomethylation	Upregulated	PCOS	[82]
	LINE-1 (5′-UTR)	Hypomethylation	NA	PCOS	[83]
	NCOR1	Hypomethylation	Upregulated	PCOS hyperandrogenism	[84]
	PPARG1	Hypermethylation	Downregulated	PCOS hyperandrogenism	
	AKR1C3	Hypomethylation	Upregulated	PCOS	[12]
	CASR	Hypomethylation	Upregulated	PCOS	
	GHRHR	Hypomethylation	Upregulated	PCOS	
	RETN	Hypomethylation	Upregulated	PCOS	
	MAMLD1	Hypomethylation	Upregulated	PCOS	
	TF	Hypomethylation	Downregulated	PCOS	
	TNF	Hypermethylation	Downregulated	PCOS	
	CD9	Hypomethylation	Upregulated	PCOS	[85]
	NR4A1	Hypomethylation	Upregulated	PCOS	
	EDN2	Hypomethylation	Upregulated	PCOS	
	BNIP3	Hypomethylation	Downregulated	PCOS	
	LIF	Hypomethylation	Upregulated	PCOS	
	YAP1	Hypomethylation	Upregulated	PCOS	[86]
Ovarian tissue	*SLC2A8*	Hypermethylation	NA	PCOS	[87]
	NRIP1	Hypermethylation	NA	PCOS	
	IGF2BP2	Hypermethylation	NA	PCOS	
	CYP19A1	Hypermethylation	Downregulated	PCOS	[87, 88]
	AMHR2	Hypermethylation	NA	PCOS	[87]
	INSR	Hypomethylation	NA	PCOS	
	AMH	Hypomethylation	NA	PCOS	
	TNIK	Hypermethylation at the CG10180092 site	Upregulated	PCOS	[89]
Cumulus cells	LINE-1 elements	Hypermethylation	Upregulated*	PCOS	[90]
	LHCGR	NA	Upregulated	Embryonic viability	[91]
	TNIK	NA	Upregulated	Embryonic viability	
	SOCS3	NA	Downregulated	Embryonic viability	
	CYP19A1	Hypomethylation	Upregulated	PCOS	[92]
Peripheral Blood	LINE-1 (5'-UTR)	Hypomethylation	NA	PCOS	[83]
	LHCGR	Hypomethylation	NA	PCOS	[82]
	LMNA CpG island	Hypermethylation	NA	PCOS-associated insulin resistance	[93]
	EPHX1	Hypomethylation	NA	PCOS	[94]
Blood Leukocytes	*PPARGC1A*	Hypermethylation	NA	PCOS metabolic risk	[95]
Adipose tissue	*LHCGR*	Hypomethylation	Upregulated	Non-obese PCOS	[96]
	INSR	Hypermethylation	Downregulated	Obese PCOS	
MicroRNAs					
Ovarian tissue	has-miR-92a	-	Downregulated	PCOS	[97]
	hsa-miR-92b	-	Downregulated	PCOS	
	hsa-miR-93	-	Upregulated	PCOS	[98]
Adipose tissue	hsa-miR-93	-	Upregulated	PCOS insulin resistance	[99, 100]
	hsa-miR-133	-	Upregulated	PCOS insulin resistance	[100]
	hsa-miR-223	-	Upregulated	PCOS insulin resistance	
Follicular Fluid	hsa-miR-132	-	Downregulated	PCOS	[101]
	hsa-miR-320	-	Downregulated	PCOS	
	hsa-miR-32	-	Upregulated	PCOS	[102]
	hsa-miR-34c	-	Upregulated	PCOS	
	hsa-miR-135a	-	Upregulated	PCOS	
	hsa-miR-18b	-	Upregulated	PCOS	

Table 2 (continued)

Cell type	Gene	Methylation status	Gene expression	Biomarker to	Reference
	hsa-miR-9	-	Upregulated	PCOS	
Serum	hsa-miR-222	-	Upregulated	PCOS	[103]
	hsa-miR-146a	-	Upregulated	PCOS	
	hsa-miR-30c	-	Upregulated	PCOS	
Whole blood	hsa-miR-21	-	Upregulated	Obese PCOS	[104]
	hsa-miR-27b	-	Upregulated	Obese PCOS	
	hsa-miR-103	-	Upregulated	Obese PCOS	

*Upregulation of genes containing LINE-1 elements, *NA*, not available

with fasting plasma glucose levels but not with total testosterone or insulin resistance [115].

Disruption of epigenetic control of gene expression through embryonic development, postnatal life, and adulthood is one of the main alterations behind the pathophysiology of complex diseases. Considering the multifaceted clinical manifestations of PCOS, an intricate network of interactions contributes to each different clinical manifestation that may have an epigenetic signature. However, each cell type presents its specific epigenetic programming, which is stably maintained during cell growth. Nevertheless, microenvironmental alterations in the ovary may change the epigenetic landscape of reproductive cells and may be related to chronic anovulation and infertility in PCOS.

Aberrant Epigenetic Reprogramming in Oocytes, Cumulus, and Granulosa Cells

Although the search for biomarkers in peripheral blood is important, the majority of studies have focused on female reproductive cells, such as oocytes, granulosa cells, and cumulus cells, since the main outcome of PCOS disorder is chronic ovulation and infertility. Increased DNA methylation level in the LINE-1 5'-UTR was observed in granulosa-cumulus cells, which was consistent with the results observed in blood cells [83]. Additionally, Pruksananonda et al. (2016) also reported increased DNA methylation levels in the LINE-1 in cumulus cells of mature oocytes from PCOS. LINE-1 hypomethylation downregulates the genes containing this element, while LINE-1 hypermethylation leads to an increase in gene expression profiles in cumulus cells [90]. Granulosa cells from PCOS women with hyperandrogenism showed hypermethylation in two CpG sites of *PPARG1* and hypomethylation at five CpG sites in the *NCOR1* promoter region. These results were consistent with the decrease in *PPARG1* expression and increase in *NCOR1* and *HDAC3* mRNA levels [84].

Altered DNA methylation patterns in *AKR1C3*, *GHRHR*, *MAMLD1*, *RETN*, and *TNF* genes in granulosa cells indirectly contribute to excessive androgen levels in PCOS patients [12]. Furthermore, a genome-wide DNA methylation study comparing epigenetic changes in ovarian granulosa cells of women with polycystic ovaries versus normal ovarian morphology, identified 106 differentially methylated CpG sites associated with 88 genes, several of which are involved in endocrine, metabolic, and reproductive processes related to PCOS. In addition, 16 of the identified CpGs were mapped within six known PCOS susceptibility loci (*YAP1*, *RAB5B/SUOX*, *HMGA2*, *KRR1*, *INSR*, *and SUMO1P1*). Of these, five overlapped with known methylation quantitative trait loci (meQTLs) [116].

GWAS provide valuable data on the origins and progression of PCOS by identifying possible targets associated with the disease. Differential expression of 59 genes has been reported in cumulus cells from PCOS women; among them, important genes for embryogenesis, angiogenesis, and sexual development, such as *LHCGR*, *ANGPTL1*, and *TNIK*, showed increased expression, whereas genes related to adipogenesis, placental development, and adult uterine morphology and function, such as *GRIN2A*, *SFRP4*, and *SOCS3*, were downregulated [91]. Subsequently, loss of methylation in the promoter region of the *LHCGR* was identified in the peripheral blood and granulosa cells of PCOS women [82]. As the genes *LHCGR*, *TNIK*, and *SOCS3* are related to the development of the embryo until the blastocyst stage, they were raised as possible biomarkers for PCOS embryonic viability [91].

Histone modifications are covalent posttranslational epigenetic modifications that alter the chromatin structure and consequently the gene expression. Histone proteins are responsible for packing the DNA into a small structure called nucleosome in the nucleus. Nucleosomes are primary chromatin subunits composed of a histone core, an octameric structure with two copies each of the histone proteins H2A, H2B, H3, and H4, wrapped with DNA and a linker histone (H1). Chemical modifications in histone tails, such as acetylation, methylation, phosphorylation, ubiquitylation, and sumoylation, modify chromatin accessibility to transcription factors, thereby affecting gene transcription and important cellular phenotypes [117]. Errors in histone posttranslational marks have been implicated in the pathogenesis of human disorders.

However, this mechanism is poorly understood in PCOS. Hosseini et al. (2019) reported increased global MeCP2 (methyl-CpG binding protein 2) and a decrease in H3K9me2 (histone H3, lysine 9 di-methylation), which can both activate and repress transcription, and increased H3K9ac (histone H3, lysine 9 acetylation), which is associated with active transcription in cumulus cells of PCOS [92].

Epigenetic changes caused by excess androgen levels are also observed in mice with PCOS induced by dehydroepiandrosterone (DHEA), in which the oocytes show decreased polar corpuscle extrusion rates, increased oxidative stress, and abnormal morphology. Furthermore, reduction in global DNA methylation and dimethylation (me2) of histone H3 on lysine 9 (H3K9), besides an increase in H4K12 acetylation, is also observed in oocytes. Consistently, DNA methyltransferase-1 (*DNMT1*) and histone deacetylase-1 (*HDAC1*) gene expression decreased in these animals [118].

Disrupted ncRNA expression was also reported in cumulus cells from PCOS women. Fifty-nine microRNAs (miRNAs) were differentially expressed in PCOS women compared to those in non-PCOS controls, in which 21 miRNAs were overexpressed and 38 were downregulated. Many of these ncRNAs are involved in important pathways related to the clinical manifestations of PCOS, including hormonal regulation, energetic metabolism, and the Notch signaling pathway that regulates cell proliferation and differentiation and is involved in follicular growth and maturation [119]. In cumulus cells, 623 long ncRNAs (lncRNAs) were differentially expressed between PCOS and non-PCOS women (five were validated using qPCR: XLOC_011402, ENST00000454271, ENST00000433673, ENST00000450294, and ENST00000432431) [120].

The lncRNA *PWRN2* plays an important role in oocyte nuclear maturation and is upregulated in PCOS cumulus cells;

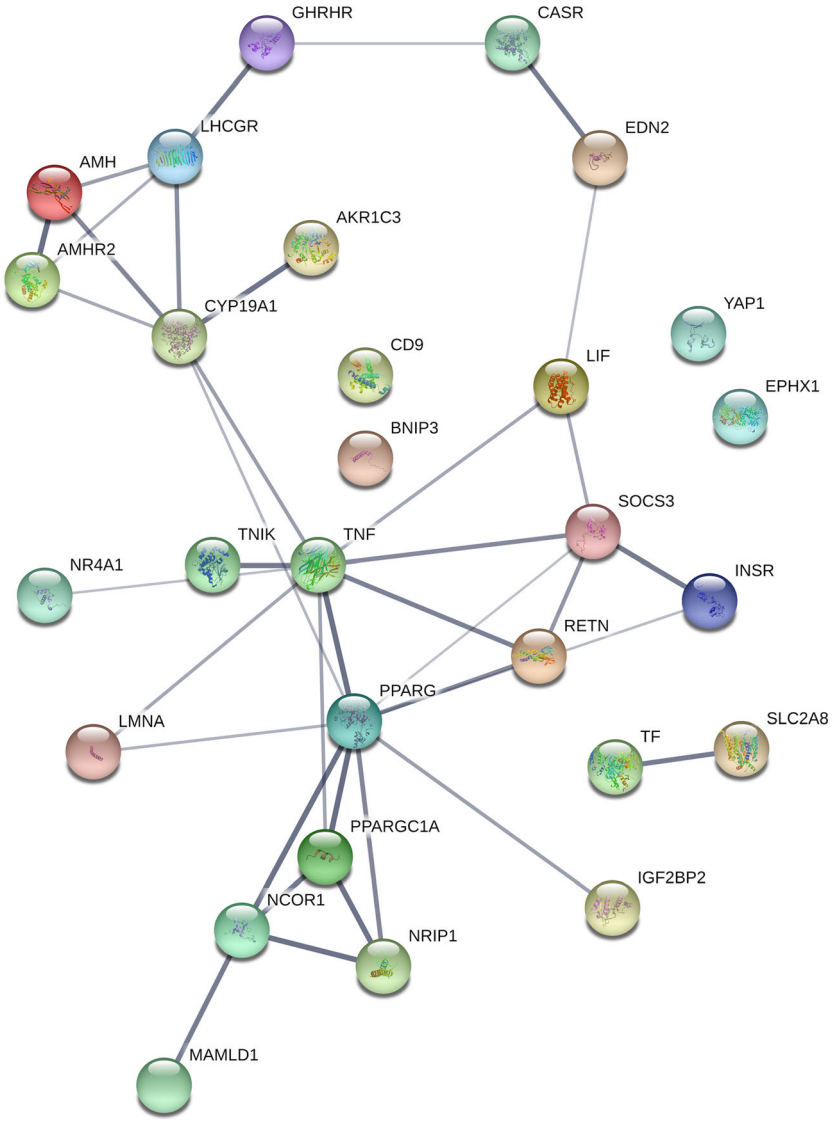

Fig. 1 STRING protein–protein interactions (PPI) network based on PCOS coding biomarkers reported. The line thickness indicates the strength of data support

Table 3 The main five functional enrichments in the PCOS-interacting proteins network based on the lowest false discovery rate

# Term ID	Term description	Count in gene set	False discovery rate
Biological process (GO)			
GO:0070887	Cellular response to chemical stimulus	21 of 2672	6.82e−10
GO:0010033	Response to organic substance	21 of 2815	9.59e−10
GO:0009719	Response to endogenous stimulus	16 of 1353	2.77e−09
GO:0048608	Reproductive structure development	11 of 405	2.77e−09
GO:0042221	Response to chemical	23 of 4153	3.73e−09
Molecular function (GO)			
GO:0005102	Signaling receptor binding	13 of 1513	1.25e−05
GO:0016500	Protein–hormone receptor activity	3 of 15	0.00029
GO:0042562	Hormone binding	4 of 90	0.00085
GO:0016922	Nuclear receptor binding	4 of 111	0.0014
GO:0030331	Estrogen receptor binding	3 of 42	0.0020
KEGG pathways			
hsa04913	Ovarian steroidogenesis	4 of 49	9.49e−05
hsa04931	Insulin resistance	4 of 107	0.00092
hsa04930	Type II diabetes mellitus	3 of 46	0.0016
hsa04920	Adipocytokine signaling pathway	3 of 69	0.0037
hsa04211	Longevity regulating pathway	3 of 88	0.0050

*GO, gene ontology

it may also be involved in the abnormal oocyte development commonly observed in PCOS [121]. Liu and colleagues (2017) showed 862 lncRNAs that were differentially expressed and the upregulation of the lncRNA *HCG26* in granulosa cells from PCOS that was associated with antral follicle count [122]. Additionally, Zhao et al. (2019) found that the expression levels of lncRNA *RP11-151A6.4* increased in granulosa cells from PCOS and suggested that this lncRNA might play an important role in IR, excess androgen, and adipose dysfunction [123]. These results were consistent with the increased expression of lncRNA *LINC-01572:28* in PCOS granulosa cells, and this upregulation was associated with hyperandrogenism [124]. Moreover, lncRNA-ovarian cancer associated 1 (*Lnc-OC1*) was overexpressed in granulosa cells from PCOS. In a steroidogenic human granulosa-like tumor cell line (KGN), Lnc-*OC1* knockdown inhibited cell viability, promoted apoptosis, and increased aromatase mRNA levels and estradiol biosynthesis. In PCOS mice, *Lnc-OC1* promoted serum insulin release, production of angiogenesis-related factors, and IκBα phosphorylation, which was partially restored after knockdown with *Lnc_OC1* shRNA [125].

Epigenetic Markers in Ovary and Follicular Fluid

Similar to oocytes and cumulus cells, the ovary also exhibits many epigenetic alterations that may compromise its morphology and viability. The analysis of more than 450,000 CpG sites and gene expression in the ovaries of PCOS women demonstrated 7929 CpG sites with differential methylation patterns and 650 differentially expressed transcripts, of which 54 genes showed altered expression related to DNA methylation changes [110]. High levels of DNA methylation in CpG island shores (regions flanking the CpG islands) and lower DNA methylation in the gene body were observed in PCOS women. In addition, the promoter regions of *SLC2A8*, *NRIP1*, *IGF2BP2*, *CYP19A1,* and *AMHR2* showed increased DNA methylation, whereas the promoters of *INSR* and *AMH* were hypomethylated [87]. In accordance, *CYP19A1* increased promoter methylation and reduced gene expression in the ovaries of PCOS women [88].

Sensitivity to epigenetic modifications may be so extreme that even a single differentially methylated CpG site may alter gene expression. PCOS women present hypermethylation at site cg10180092, a critical site in the regulation of *TNIK* transcription and a decreased H3K9me level, a repressive histone modification [110], leading to increased *TNIK* expression in PCOS ovarian tissue. This is a peculiar case in which a single point DNA hypermethylation affects histone methylation, thereby altering *TNIK* gene expression [89]. In contrast, epigenetic alterations may often not reflect differences in gene expression; for example, ovarian tissue from testosterone-induced PCOS mice showed hypomethylation at five sites in the *AR* gene promoter region and one site on the *Cyp11a1* gene promoter. However, these differences were not sufficient to alter the expression of these genes [126].

MiRNAs also exert influence on the ovaries. Lin et al. (2015) identified 27 miRNAs differentially expressed in the ovaries of PCOS women compared to those in the controls. The downregulation of miR-92a and miR-92b in PCOS was confirmed using qPCR [97]. Increased expression of miR-93 and its target *CDKN1A* gene was downregulated in ovarian cortex immortalized granulosa cells, which might be due to the high concentration of insulin, a common condition in PCOS women [98].

The miRNAs in the follicular fluid (microvesicles and supernatant) and the overexpression of target genes in PCOS women are associated with reproduction, endocrine system, and metabolic processes [101, 102]. A positive relationship was observed among miR-132, miR-320, miR-520c-3p, miR-222, and estradiol concentrations, while a negative relationship was noted among miR-24, miR-193b, miR-483-5p, and progesterone concentrations [101]. In PCOS women, miR-132 and miR-320 are expressed at lower levels, whereas miR-32, miR-34c, miR-135a, miR-18b, and miR-9 show increased expression in the follicular fluid of PCOS [101, 102]. The lncRNA profile in follicular fluid from mature and immature ovarian follicles also differs between the PCOS and controls, and lncRNAs related to the metabolic process are highly enriched in the PCOS mature follicular fluid [127].

Mitochondrial Dysfunctions

Besides genetic, epigenetic, and environmental factors, mitochondrial dysfunction may play a role in the pathogenesis of PCOS. Ding et al. (2017) identified nine homoplasmic variants in mt-tRNAs that were associated with PCOS women with IR: mt-tRNA$^{Leu(UUR)}$ A3302G and C3275A, mt-tRNA^{Gln} T4363C and T4395C, mt-tRNA$^{Ser(UCN)}$ C7492T, mt-tRNA^{Asp} A7543G, mt-tRNA^{Lys} A8343G, mt-tRNA^{Arg} T10454C, and mt-tRNA^{Glu} A14693G. These mutations alter the secondary structure of mt-tRNAs, affecting mt-tRNA metabolism, thereby increasing the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde, and reactive oxygen species (ROS) in PCOS-IR women [128].

A previous study identified 16 variants in the noncoding region D-Loop, seven variations in the 12S rRNA gene, three variants in the 16S rRNA gene, and several variants of the oxidative phosphorylation (OXPHOS) complex in PCOS women [129]. The study also identified six variants in mitochondrial tRNA genes, including tRNA^{Gln}, tRNA^{Cys}, tRNA^{Asp}, tRNA^{Lys}, tRNA^{Arg}, and tRNA^{Glu}, localized in conserved regions important for tRNA stability and biochemical function [129]. Therefore, it may be possible that the cumulative effect of these mutations contributes to mitochondrial dysfunction, thereby contributing to PCOS pathogenesis.

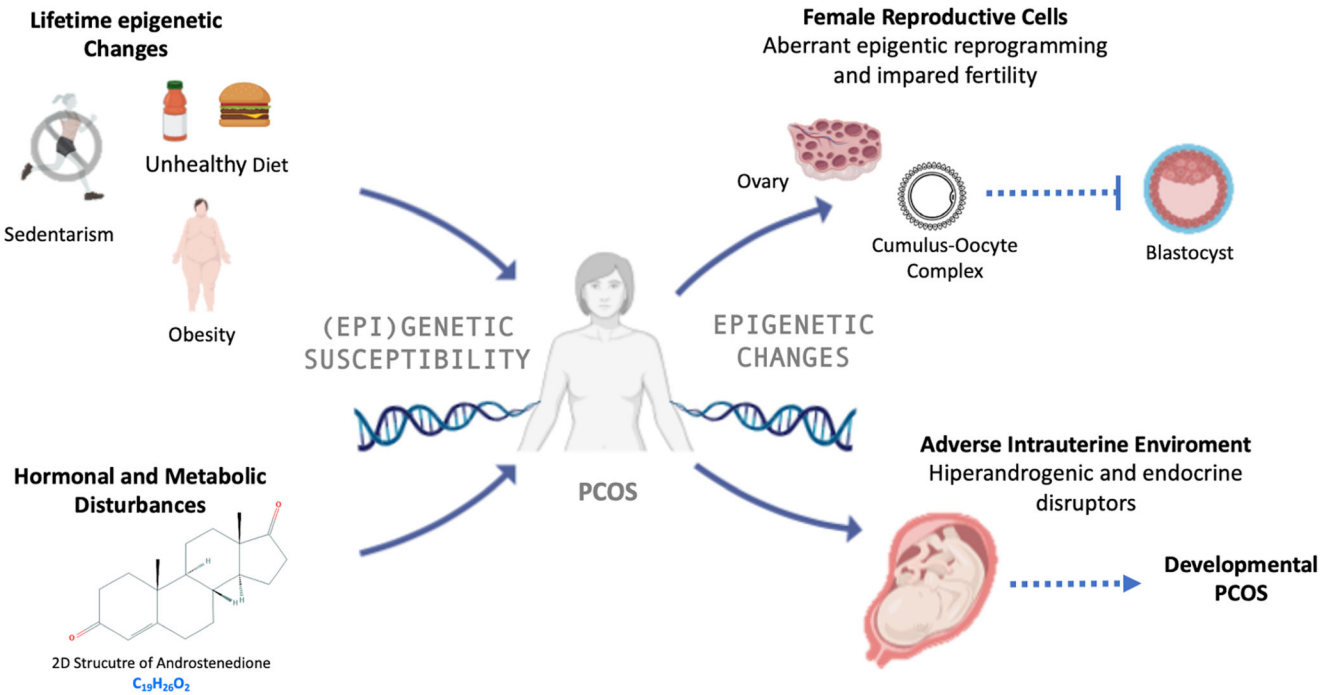

Fig. 2 Lifestyles changes as unhealthy diet, sedentarism, and obesity, as well as hormonal and metabolic disturbances are environmental factors that combined with a genetic predisposition (candidate genes) changes epigenome landscape leading to (epi)genetic susceptibility to PCOS development throughout life. On the other hand, the hormonal and endocrine disturbances in PCOS directly affect folliculogenesis and gametogenesis, resulting in chronic anovulation, impaired fertilization, reduced implantation rates, and miscarriage. These clinical alterations affect embryo development since intrauterine environmental changes as hyperandrogenism, nutritional imbalance, and metabolic syndrome impact the epigenetic reprograming during fetal life that may trigger developmental PCOS or its associated clinical alterations after birth

Moreover, analysis of the mitochondrial displacement-loop (D-loop) of Indian PCOS women and the controls showed a significant association between D310 and A189G SNPs with PCOS, resulting in lower mtDNA copy number for patients carrying D310 and 189G alleles, and an elevated LH/FSH ratio for D310 carriers [130]. Mitochondrial D-loop is a hotspot region for mtDNA mutations; these mutations can interrupt the sequence in the promoter region, thereby altering the affinity for inducers/modifiers of mtDNA replication and/or transcription, which may further affect the electron transport chain machinery and consequently, cellular ROS generation [130].

Mutation analysis of the whole mitochondrial genes in a three-generation pedigree with maternally transmitted metabolic syndrome, combined with PCOS, identified multiple variants, especially in mt-tRNA genes ($tRNA^{Leu(UUR)}$ C3275T, $tRNA^{Gln}$ T4363C, and $tRNA^{Lys}$ A8343G). These mutations altered mt-tRNA metabolism, resulting in a deficiency in mitochondrial functions (lower levels of MMP, ATP production, and mtDNA copy number, and increase in ROS generation), which could be responsible for the clinical phenotypes [131].

Variants in nuclear-encoded genes regulating mitochondrial biogenesis, such as *TFAM* and *PGC-1α*, were also associated with PCOS, leading to reduced mtDNA copy number and higher levels of the luteinizing (LH) hormone [132]. Mitochondrial dysfunctions in the peripheral blood, leukocytes, follicular fluid, skeletal muscle cells, cumulus cells, and endometrial cells from PCOS women have also been described in several studies [133–136].

Conclusions and Perspectives

PCOS is an ancient, heterogeneous, multifactorial, and complex disorder; understanding the mechanisms underlying the pathophysiology of this syndrome and the consequences for female fertility, is challenging. The diversity of clinical manifestations observed in PCOS suggests that a combination of multiple factors is related to the pathophysiology of this syndrome (Fig. 2). Genetic predisposition (candidate genes) and a strong environmental contribution owing to lifestyle changes, obesity, and hormonal and metabolic disruptors lead to (epi)genetic susceptibility to PCOS development throughout life. Nevertheless, hyperandrogenism and metabolic disorders directly affect ovary morphology and folliculogenesis, which may alter the epigenetic landscape of reproductive cells, leading to chronic anovulation, impaired fertility, reduced embryo quality, and miscarriage. Environmentally induced epigenetic changes caused by an intrauterine adverse environment, as hyperandrogenism during early embryo development may trigger developmental PCOS or its associated clinical alterations after birth.

However, the clinical alterations of PCOS and its associated health conditions have a great plasticity, changing or even disappearing throughout a lifetime. Lifestyle changes, such as physical activity and a healthy diet, directly affect metabolic disorders and improve fertility in PCOS through epigenetic changes in gene expression. The identification of possible molecular markers and epigenetic modifications related to the different phenotypes observed in PCOS, such as IR, hyperandrogenism, chronic anovulation, obesity, and other related alterations, could direct the treatment and improve the quality of life and fertility of women with PCOS. However, the incidence of PCOS is growing and many aspects of this syndrome and its effects on female fertility and quality of life remain unclear. Therefore, accurate investigation of the epigenetic mechanisms underlying this syndrome is of utmost importance for advancing the understanding and treatment of PCOS.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43032-021-00516-3.

Acknowledgements We would like to thank the members of the Human Reproduction Division at Department of Gynecology and Obstetrics of the Ribeirao Preto Medical School, University of Sao Paulo (FMRP-USP), and the members of the Laboratory of Experimental Oncology (LOE) at Drug Research and Development Center, Federal University of Ceara (NPDM-UFC). Fig. 2 was made in part using *BioRender*. The English was revised by *Editage*.

Funding This study was supported by the Sao Paulo Research Foundation (FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo) with the grant 2015/14031-0 (RMR) and fellowship 2012/11069-9 (CLMF), National Council for Scientific and Technological Development (CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico), Coordination for the Improvement of Higher Education Personnel (CAPES, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Academic Excellence Program - PROEX and Graduate Support Program - PROAP) and by the National Institutes of Science and Technology (INCT, Institutos Nacionais de Ciência e Tecnologia).

Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Ethics Statement Since this is a review article any human or animal samples were used in this manuscript, so no ethical consents are required.

Consent to Participate Not applicable.

Consent for Publication All authors approved the final version and consent to the publication of this study.

Conflict of Interest The authors declare no competing interests.

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