Archives of Gynecology and Obstetrics (2024) 309:373–383
https://doi.org/10.1007/s00404-023-07025-5

REVIEW

DNA methylation associated with polycystic ovary syndrome: a systematic review

Arícia Gomes Miranda[1] · **Marcelo Marcondes Seneda**[2] · **Luciana Rocha Faustino**[1]

Received: 9 July 2022 / Accepted: 8 March 2023 / Published online: 29 April 2023

Abstract

Purpose Polycystic ovary syndrome (PCOS) is an endocrine metabolic disease that affects women of reproductive age and is one of the main causes of anovulatory infertility. However, the cause of PCOS is yet fully understood, and genetic factors play an important role in its etiology. In this study, we reviewed the main genes involved in the etiology of PCOS and the influence of DNA methylation, aiming to answer the study´s guiding question: 'What is the influence of DNA methylation on the main genes involved in PCOS?'.

Methods We used the MEDLINE database, and inclusion criteria (primary and original articles, written in English, found through our entry terms) and exclusion criteria (literature reviews and articles that used animals to perform the experiments and that focused in other epigenetics mechanism without being DNA methylation) were applied.

Results Twenty-three scientific articles, from a total of 43 articles read in full, were chosen for this study. Eighteen studies confirmed DNA methylation associated with PCOS.

Conclusion The most relevant genes related to PCOS were *INSR*, *LHCGR*, and *RAB5B*, which may be epigenetically altered in DNA, with the first two genes hypomethylated and the last hypermethylated. The epigenetic changes presented in the genes related to PCOS or their promoters were only at the CpG sites.

Keywords Infertility · Genes · DNA · Methylation

What does this study add to the clinical work

This systematic review on the main genes related to the PCOS physiopathology lead to a deeper understanding of the PCOS pathogenesis, and have the potential to orientate more precise diagnoses, and also help to establish more effective treatment protocols.

Introduction

Polycystic ovary syndrome (PCOS) is an endocrinological disease that affects 6–15% of women of reproductive age [1]. This syndrome has a combination of symptoms, such as hyperandrogenism, menstrual irregularities, metabolic syndrome, infertility, acne, and obesity [2]. According to the Rotterdam Consensus Workshop criteria for PCOS [3], it is necessary to identify at least two of the following characteristics: (i) clinical or biochemical signs of hyperandrogenism, (ii) oligo or anovulation, and (iii) presence of polycystic ovaries.

In addition to the heterogeneity of clinical signs and symptoms, PCOS has an etiology that is not fully understood. Thus, the study of this disease is quite challenging [1], being the object of an increasing number of studies over the years (Fig. 1). Among the possible causes of the development of PCOS, the excess production of androgens and insulin resistance have been identified as the main factors in the etiology of the disease [4].

✉ Luciana Rocha Faustino
lrfaustino@gmail.com

[1] Curso de Medicina, Campus Ministro Reis Velloso, Universidade Federal do Delta do Parnaíba (UFDPar), Parnaíba, Piauí, Brazil

[2] Laboratório de Reprodução Animal, Centro de Ciências Agrárias, Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brazil

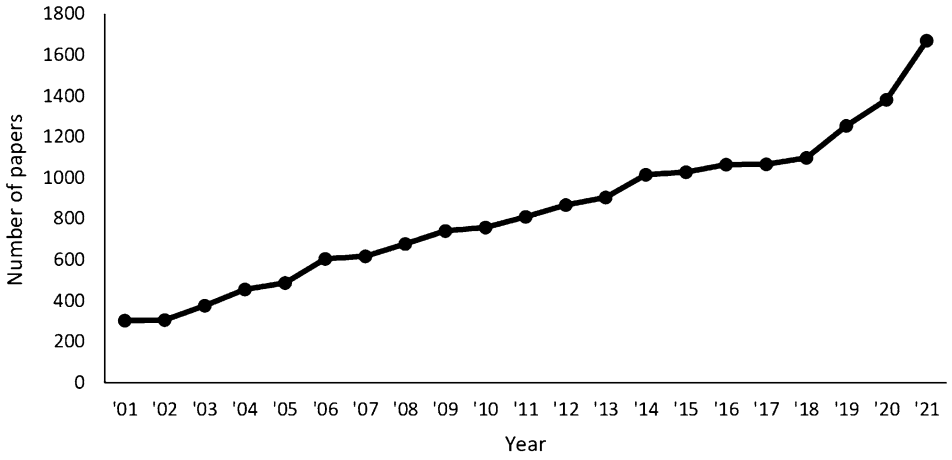

Fig. 1 Survey of the number of articles on polycystic ovary syndrome (PCOS), using the MEDLINE database. Over the years, it is possible to notice the increase in the number of articles on PCOS, pointing to a greater number of researches, knowledge and relevance of the subject. This chart is created in late June 2022

Genetic factors also play an important role in the etiology of the disease, as alterations in gene transcription or genetic polymorphisms can cause serious transcriptional alterations related to PCOS. According to Ajmal et al. [5], the genes that encode androgen receptors, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and leptin receptors are the most likely to be involved in the pathophysiology of the disease.

Epigenetics, which deals with processes associated with changes in the expression pattern of genes without changing the DNA nucleotide sequence [6], is a branch of genetics that is increasingly associated with the pathogenesis of PCOS [7]. Epigenetic processes include DNA cytosine methylation, modifications of histone proteins present in the nucleosome, and mechanisms mediated by noncoding RNA [8]. DNA methylation involves the addition of a methyl group on carbon 5 of cytosine through the action of DNA methyltransferases [9, 10]. Much of this methylation occurs at CpG sites, which are groups of dinucleotides, resulting in chromatin condensation. Therefore, hypermethylated DNA regions hinder gene transcription and cause gene silencing [9]. In histones, several covalent modifications can occur, such as acetylation, methylation, phosphorylation, and ubiquitination [10, 11], which change the conformation and accessibility of chromatin in different ways [11]. Noncoding RNAs, in turn, are transcribed from RNAs that do not code for proteins but can, for example, interact with histone-modifying complexes or DNA methyltransferases to regulate gene expression [12].

In addition to being current, the relationship between epigenetics and PCOS is quite relevant, as changes in gene expression can generate important phenotypic changes, such as hyperandrogenism [7, 13–15]. Thus, the discovery and investigation of genes undergoing epigenetic alterations in tissues affected by the pathology may lead to more effective therapies for the treatment of women with PCOS [7, 13–15]. However, given the clinical heterogeneity of PCOS associated with the complex gene expression pathways involved in this disease, there are still gaps to be filled regarding its etiology. Therefore, this study aimed to review the main genes involved in the pathophysiology of PCOS and DNA methylation associated with the expression of these genes.

Methods

This study is a systematic review that used the updated Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendation [16] (Fig. 2). The study's guiding question was: "What is the influence of DNA methylation on the main genes involved in PCOS?".

The search was carried out until June 2022 in the Medical Literature Analysis and Retrieval System Online (MEDLINE) database. The descriptors used for the search were (polycystic ovary syndrome) AND (genes) AND (epigenetics) based on the MeSH descriptors.

We included papers that met our inclusion criteria: primary and original articles, written in English, found through our entry terms. All papers included must have been published until June 2022. Our exclusion criteria were papers written in other language than English, literature reviews, and articles that used animals to perform the experiments or that focused in other epigenetics mechanism instead of DNA methylation.

For data screening and extraction, a table was filled with the quantification of the following data: author, year of publication, cited genes, methods, objectives, main genes addressed in each study, and type of epigenetic alteration involved in the expression of these genes when epigenetically altered. To be included for data screening and extraction, the paper must have analyzed the DNA of women with and without PCOS. Our primary outcome measure was the genes differentially expressed in these two groups, and

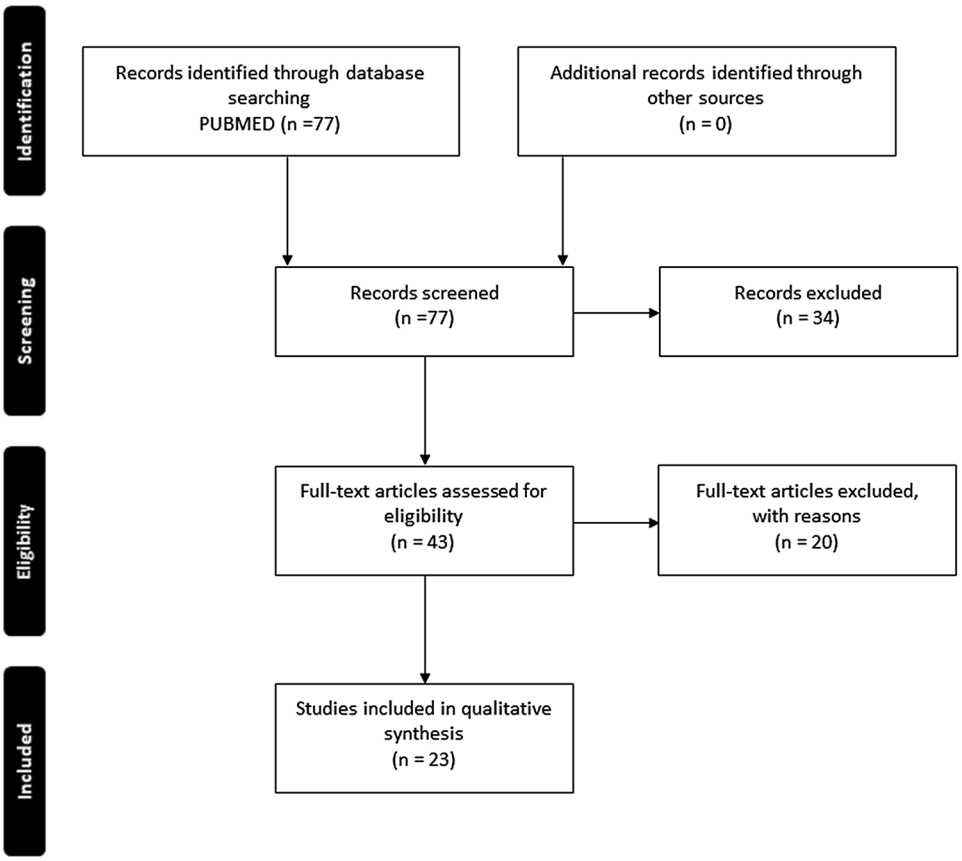

Fig. 2 Flowchart of the systematic review of manuscripts related to epigenetic alterations of genes involved in polycystic ovary syndrome (PCOS), based on the PRISMA recommendation. The records are identified by searching the MEDLINE database of articles published until December 2021. After applying the exclusion criteria, 77 manuscripts are selected. After a complete reading of 43 articles, 23 articles are in accordance with the study proposal and, therefore, are included in this review

this difference had to be explained by the methylation levels. Then, articles were grouped based on the similarity of the genes and their metabolic role. The data selection and extraction were done and reviewed by two authors independently. Subsequently, a third author reviewed the results and pointed out suggestions. Only the papers that met the inclusion criteria were added to this systematic review, and any inter-researched disagreement was resolved among the authors. The risk of bias in included papers was assessed by the Newcastle–Ottawa Scale (NOS) [17], with modifications (Supplementary Table 1). The NOS measures the quality of nonrandomized studies based on the selection of the study groups, the comparability of the groups, and the ascertainment of either the exposure or outcome of interest for case–control or cohort studies, respectively, to be used in a systematic review [17]. This scale allowed us to evaluate all the articles with the same tool, as not all of them were case–control studies. The criteria adopted were: (1) adequate definition of cases; (2) selection of controls; (3) control for important factor; (4) explicit DNA tissue extracted reported; and (5) significant statistic difference between PCOS *vs* control for DNA methylation levels.

Results

In total, we identified 77 articles. After screening based on the title or abstract, 34 studies were excluded. Among these, 19 were reviews, five focused on miRNA modifications, and 10 did not focus on epigenetic modifications. Forty-three articles were read in full, and 20 papers were excluded because studied epigenetic alterations in a non-human population. Finally, 23 were eligible to answer the central question of this review. We summarized this process in a flow-diagram (Fig. 2). The studies appraisal was qualitatively done by stratifying methodological characteristics of each study, i.e., the type of study, the size of the included population, the presence of a control group, the genes involved, and their methylation and expression levels. Then, the quality of the assessment of the included studies was measured by NOS (Supplementary Table 1).

Among the included articles, the main genes affected by PCOS were identified and are detailed in Table 1. The genes for androgen receptors and those related to the regulation of ovulation and metabolism were the most recurrent.

Of the 23 selected articles, 18 confirmed the epigenetic influence on PCOS-related genes. Table 2 lists those with greater relevance to the epigenetic alterations described by the authors, with genes identified in more than one study.

Table 1 List of the main genes affected in polycystic ovary syndrome (PCOS), according to a literature review

Gene	Function	Authors
AKR1C3	Associated with hyperandrogenism as it converts A4 into testosterone. It is also related to premature rupture of the luteum and luteal insufficiency	Sagvekar et al. (2019) [28]
ADIPOQ	Involved in the functionality of adipocytes	Leung et al. (2020) [22]
	Responsible for the production of adiponectin in adipocytes. Related to androgen dysfunction in childhood children whose mothers have a PCOS	Echiburú et al. (2020) [29]
AFAP1	NR	Wang et al. (2014) [32]
AGPAT2	Involved in the differentiation of adipocytes	Leung et al. (2020) [22]
AIFM1	NR	Wang et al. (2014) [32]
AMH	Plays a key role in folliculogenesis	Echiburú et al. (2020) [29]
	Regulates follicular recruitment and selects cyclically anti follicles	Yu et al. (2015) [24]
ANKRD11	NR	Wang et al. (2014) [32]
APP	Detrimental to mitochondrial metabolism and possibly responsible for increased oxidative stress	Lambertini et al. (2017) [25]
AR	Androgens receiver	Dasgupta et al. (2010) [18] Laisk et al. (2010) [19] Echiburú et al. (2020) [29]
ARHGEF26	Endocrine function	Makrinou et al. (2020) [30]
ATF1	Ovarian function	Makrinou et al. (2020) [30]
BDNF	Encodes a protein found in the brain that promotes the survival of neurons. Related to the level of glucose, type 2 diabetes melitus, and insulin sensitivity	Jacobsen et al. (2019) [27]
BMPR1A	Ovarian function	Makrinou et al. (2020) [30]
BNIP3	Related to lipid metabolism	Pan et al. (2018) [33]
BRCA1	Associated with breast cancer and possibly ovarian	Jiao et al. (2019) [39]
CARNMT1	Metabolism of lipids, cholesterol, and adipogenesis	Makrinou et al. (2020) [30]
CASP9	Ovarian function	Makrinou et al. (2020) [30]
CASR	Its expression affect the signage pathways of CA2 +, which impairs oocyte maturation or causes poor oocytes production	Sagvekar et al. (2019) [28]
CCL2	NR	Wang et al. (2014) [32]
CEBPα	Involved in the differentiation of adipocytes	Leung et al. (2020) [22]
CHRNA4	Related to neurotransmitters and mental disorders	Makrinou et al. (2020) [30]
CNN1	Ovarian function	Makrinou et al. (2020) [30]
COX15	Related to the cardiovascular system	Makrinou et al. (2020) [30]
CREM	NR	Wang et al. (2014) [32]
CYP19A1	Responsible for encoding aromatase, a key enzyme that catalyzes the final stage of estrogen biosynthesis	Yu et al. (2015) [24]
CYP4X1	NR	Wang et al. (2014) [32]
DCAF12L1	NR	Wang et al. (2014) [32]
DDB2	NR	Wang et al. (2014) [32]
DHRS9	NR	Xu et al. (2016) [13]
DIP2C	NR	Wang et al. (2014) [32]
DIRAS3	NR	Saenz-de-Juano et al. (2019) [21]
DLG1	Ovarian function. Related to neurotransmitters and mental disorders	Makrinou et al. (2020) [30]
DNAJC5	NR	Wang et al. (2014) [32]
ESR1	Related to metabolic dysfunction, such as dyslipidemia	Lambertini et al. (2017) [25]
EXPH5	Metabolism of insulin	Makrinou et al. (2020) [30]
FABP4	Involved in the functionality of adipocytes	Leung et al. (2020) [22]
FAHD1	NR	Wang et al. (2014) [32]
FAM50B	NR	Saenz-de-Juano et al. (2019) [21]
FBLN5	Ovarian function	Makrinou et al. (2020) [30]
FBN1	NR	Wang et al. (2014) [32]
FERMT2	Ovarian and endocrine function	Makrinou et al. (2020) [30]

Table 1 (continued)

Gene	Function	Authors
FST	Encodes the production of follistatin	Sang et al. (2013) [20]
FZD1	NR	Leung et al. (2020) [22]
GADD45B	Ovarian function	Makrinou et al. (2020) [30]
GEMIN8	NR	Wang et al. (2014) [32]
GHRHR	Regulates the release of GH and IGF1. IGF1 stimulates the production of LH and that of androgens	Sagvekar et al. (2019) [28]
GM2A	Metabolism of lipids, cholesterol, and adipogenesis	Makrinou et al. (2020) [30]
GNA11	Metabolism of insulin. Ovarian and endocrine function	Makrinou et al. (2020) [30]
GNAS	NR	Saenz-de-Juano et al. (2019) [21]
GNG4	NR	Wang et al. (2014) [32]
HAPLN1	Regulation of COC	Sagvekar et al. (2019) [28]
HCG4	NR	Wang et al. (2014) [32]
HLA-B	NR	Wang et al. (2014) [32]
HLA-F	NR	Wang et al. (2014) [32]
HMGA2	NR	Makrinou et al. (2020) [30]
HOOK2	Correlated with obesity and type 2 diabetes mellitus	Jacobsen et al. (2019) [27]
HRH1	NR	Wang et al. (2014) [32]
HSD17B1	NR	Wang et al. (2014) [32]
HTR5A	NR	Saenz-de-Juano et al. (2019) [21]
IGF2BP2	Type 2 diabetes and plays a role in oocyte competence	Yu et al. (2015) [24]
INSR	Indispensable in the life of insulin signaling	
	Insulin receiver	Jones et al. (2015) [23]
IRS1	Related to insulin signaling and is also a candidate for type 2 diabetes mellitus	Kokosar et al. (2016) [15]
ITGBL1	NR	Wang et al. (2014) [32]
KCNK1	Ovarian function	Makrinou et al. (2020) [30]
KCNMA1	NR	Wang et al. (2014) [32]
KLF10	Related to the size of adipose tissue in women with PCOS	Nilsson et al. (2018) [26]
LEP	Present mainly in adipose tissue, related to BMI disorders and insulin regulation in women with PCOS	Echiburú et al. (2020) [29]
LHCGR	LH receiver	Wang et al. (2014) [32] Jones et al. (2015) [23]
LIF	Its decrease was associated with small deployment rates and low levels of success in in vitro fertilization	Sagvekar et al. (2019) [28]
LINE1	Found in large numbers in the human genome, it is related to diseases of the gynecological system: gestational trophoblastic neoplasm, endometriosis, among others	Pruksananonda et al. (2016) [34]
LPCAT1	Codifies the enzyme lisofosphatyldicoline acyltransferase (LPCAT), which plays a role in glucose metabolism	Mao et al. (2021) [31]
LPL	Involved in the functionality of adipocytes	Leung et al. (2020) [22]
LRP1	Metabolism of lipids, cholesterol, and adipogenesis. Ovarian function	Makrinou et al. (2020) [30]
MAD1L1	NR	Wang et al. (2014) [32]
MAFK	NR	Wang et al. (2014) [32]
MAMLD1	Excess androgen	Sagvekar et al. (2019) [28]
MAP2K6	Related to insulin resistance	Nilsson et al. (2018) [26]
MAPKAPK3	Related to neurotransmitters and mental disorders	Makrinou et al. (2020) [30]
MDK	Endocrine and ovarian function	Makrinou et al. (2020) [30]
MLH1	Increased risk of developing ovarian cancer	Jiao et al. (2019) [39]
MTSS1	Related to the cardiovascular system	Makrinou et al. (2020) [30]
MLXIPL	Metabolism of insulin	Makrinou et al. (2020) [30]
NAMPT	Related to a possible compensation decreased insulin sensitivity, in addition to relating to the size of the adipocytes	Nilsson et al. (2018) [26]
NAP1L5	NR	Saenz-de-Juano et al. (2019) [21]

Table 1 (continued)

Gene	Function	Authors
NAV2	NR	Wang et al. (2014) [32]
NBAS	NR	Wang et al. (2014) [32]
NCF2	NR	Xu et al. (2016) [13]
NR0B1	NR	Wang et al. (2014) [32]
NR4A1	Biosynthesis of androgens and changes in the circadian cycle	Pan et al. (2018) [33]
NRIP1	Central paper in lipid and carbohydrate metabolism. Associated with infertility	Yu et al. (2015) [24]
NRK	NR	Wang et al. (2014) [32]
PARK2	Associated with oxidative stress and brain insulin strength	Lambertini et al. (2017) [25]
PAX6	Essential for the differentiation and survival of pancreatic beta cells (responsible for producing insulin)	Lambertini et al. (2017) [25]
PCYT1A	Codifies the enzyme hill-phosphate citidyltransferase, which is important in phosphatidyl synthesis	Mao et al. (2021) [31]
PLAGL1	NR	Saenz-de-Juano et al. (2019) [21]
PLN1	Involved in the functionality of adipocytes	Leung et al. (2020) [22]
PLXNC1	NR	Wang et al. (2014) [32]
PPARG/PPARy	Related to type 2 diabetes mellitus	Kokosar et al. (2016) [15]
	Involved in the differentiation of adipocytes	Leung et al. (2020) [22]
PPARGGC1	Related to biogenesis, mitochondrial function and changes in methylation in children were related to increased cardiometabolic risk	Zhao et al. (2017) [35]
PPFIA2	Related to neurotransmitters and mental disorders	Makrinou et al. (2020) [30]
PRDM1	NR	Wang et al. (2014) [32]
PRKCA	Ovarian function	Makrinou et al. (2020) [30]
PTGER1	Decrease in the angiogenesis of ovarian endothelial cells. In addition, associated with premature luteolysis and soon recurring abortions	Sagvekar et al. (2019) [28]
RAB5B	Involved in the transport of intracellular vesicles	Kokosar et al. (2016) [15]
	Related to the formation of endosomal cells and is related to diabetes mellitus 1 and obesity	Jones et al. (2015) [23]
RASSF5	NR	Wang et al. (2014) [32]
RDX	Ovarian function	Makrinou et al. (2020) [30]
RETN	Modulates tolerance to glucose and insulin action. Related to insulin resistance and excess androgen	Sagvekar et al. (2019) [28]
RGMB	NR	Wang et al. (2014) [32]
RLIM	NR	Wang et al. (2014) [32]
RNF213	NR	Wang et al. (2014) [32]
SCGB1D4	Regulates the migration and invasion of chemotactic cells	Hiam et al. (2019) [35]
SCNA	NR	Xu et al. (2016) [13]
SFRP1	NR	Leung et al. (2020) [22]
SHH	Ovarian function	Makrinou et al. (2020) [30]
SLC2A4	Involved in the functionality of adipocytes	Leung et al. (2020) [22]
SLC2A8	Member of a family of intracellular glucose conveyors	Yu et al. (2015) [24]
SNRPN	NR	Saenz-de-Juano et al. (2019) [21]
SSBP2	NR	Wang et al. (2014) [32]
SULT4A1	Related to the cardiovascular system	Makrinou et al. (2020) [30]
STXBP5L	Metabolism of insulin	Makrinou et al. (2020) [30]
SVEP1	Related to type 2 diabetes mellitus	Kokosar et al. (2016) [15]
TGFB1	Related to chronic systemic inflammation	Lambertini et al. (2017) [25]
TGFB1	The primary regulator of genes expressed on days 3, 7, and 12 of adipogenesis	Leung et al. (2020) [22]
TMEM132A	NR	Wang et al. (2014) [32]
TNF	Related to hyperandrogenism, COC expansion, and ovulation commitment	Sagvekar et al. (2019) [28]
TNIK	NR	Wang et al. (2014) [32]
TPRG1	NR	Wang et al. (2014) [32]
TRIM14	NR	Wang et al. (2014) [32]

Table 1 (continued)

Gene	Function	Authors
WDR44	NR	Wang et al. (2014) [32]
WNT10B	NR	Leung et al. (2020) [22]
YWHAQ	NR	Xu et al. (2016) [13]
ZFAND3	NR	Wang et al. (2014) [32]
ZNF503	NR	Wang et al. (2014) [32]

A4 androstenedione, *COC* cumulus oophorus complex, *GH* growth hormone, *IGF1* insulin's similar growth factor, *BMI* body mass index, *LH* luteinizing hormone, *NR* not reported, *PCOS* polycystic ovary syndrome

The epigenetic alterations presented were only in DNA, specifically CpG sites (Fig. 3), which could be hypomethylated or hypermethylated depending on the gene. Therefore, no alterations in histones were observed. In the other articles ($n=5$), although the direct influence of epigenetics on the genes involved in PCOS was not found, no work contradicted the existence of this influence [18–22]. Among the articles that confirmed the relationship between epigenetics and PCOS genes, ten articles identified epigenetic alterations in genes related to insulin resistance [15, 23–31]. In total, 14 genes were identified: growth hormone releasing hormone receptor (*GHRHR*), peroxisome proliferator activated receptor gamma (*PPARG*), resistin (*RETN*), nicotinamide phosphoribosyltransferase (*NAMPT*), brain derived neurotrophic factor (*BDNF*), insulin receptor substrate 1 (*IRS1*), paired box 6 (*PAX6*), insulin receptor (*INSR*), G protein subunit alpha 11 (*GNA11*), MLX interacting protein like (*MLXIPL*), syntaxin binding protein 5L (*STXBP5L*), leptin (*LEP*), estrogen receptor 1 (*ESR1*), and lysophosphatidylcholine acyltransferase 1 (*LPCAT1*). Other studies also identified genes related to hyperandrogenism: luteinizing hormone/choriogonadotropin receptor (*LHCGR*), CL2 interacting protein 3 (*BNIP3*), *GHRHR*, and tumor necrosis factor (*TNF*) [23, 28, 32, 33].

Discussion

Given the high number of genes cited in the articles, many authors have not discussed their relationship with PCOS in depth. Wang et al. [36], for example, analyzed an extensive number of genes, 54 in all, to test their correlation with PCOS. However, not all genes showed a direct relationship between methylation and regulation of gene expression or function, or their function had not been well elucidated by methylation [36].

One of the main genes reported was the *INSR* gene. The promoter regions of the *INSR* gene were reported by Yu et al. [24] as hypomethylated in the ovarian tissue of women with PCOS compared with women without PCOS, resulting in a greater expression of the *INSR* gene. Jones et al. [23], in turn, verified that the *INSR* gene was expressed more in cumulus cells of ovarian follicles of obese women with PCOS than in non-obese women with PCOS. They presented another relevant finding that the *INSR* gene is hypermethylated, i.e., less expressed in metabolic tissues, such as skeletal muscle tissue, of obese women with PCOS [23]. This finding was used to confirm the existing theory of selective insulin sensitivity, since ovarian tissue is not resistant to insulin while skeletal muscle is [23].

Of the other genes that also underwent epigenetic alterations and were related to PCOS and insulin resistance, the *ESR1* gene, an androgen receptor, was found to be hypomethylated in women with PCOS compared to the control group (women without PCOS), which was overexpressed [25]. The authors associated overexpression of the *ESR1* gene with overexpression of lipid kinases related to the development of insulin resistance, which may be a possible explanation for the glucotoxic environment in women with PCOS [25]. Another important gene, *IRS1*, which is a gene that plays a central role in insulin signaling and a relationship with type 2 diabetes mellitus, was shown to be altered based on the body mass index of women [15]. The authors showed that overweight or obese women with PCOS had reduced *IRS1* expression compared to others in the cohort [15]. Similarly, another gene directly correlated with type 2 diabetes mellitus, *PPARG*, one of the targets of pharmacological drugs used to control plasma glucose levels, was found to be hypermethylated and, therefore, less expressed in women with PCOS [15]. When studying the PPARG coactivator 1 alpha (*PPARGC1A*), a *PPARG* coactivator [37], Zhao et al. observed that women with PCOS also had this hypermethylated gene compared to healthy control women [38], indicating an epigenetic orchestration of genes with integrated functions.

A review of the articles also pointed to epigenetic alterations in genes related to the regulation of ovulation, such as the *LHCGR* gene, which encodes the LH and chorionic gonadotropin (CG) receptor. In two studies, elevated expression of this gene was found in women diagnosed with PCOS compared to women without PCOS due to its hypomethylation [23, 32].

Table 2 Epigenetic alterations of genes were found in women with polycystic ovary syndrome (PCOS), according to a literature review

Gene	Epigenetic alteration	References
AKR1C3	Hypomethylated	Sagvekar et al. (2019) [28]
AMH	Hypomethylated	Echiburú et al. (2020) [29]
APP	Hypomethylated	Lambertini et al. (2017) [25]
AR	Hypomethylated	Echiburú et al. (2020) [29]
BDNF	Hypermethylated	Jacobsen et al. (2019) [27]
BMPR1A	Hypomethylated	Makrinou et al. (2020) [30]
BNIP3	Hypomethylated	Pan et al. (2018) [33]
CASR	Hypomethylated	Sagvekar et al. (2019) [28]
CCDC48	Hypermethylated	Makrinou et al. (2020) [30]
CYP19A1	Hypermethylated	Yu et al. (2015) [24]
DHRS9	Hypomethylated*	Xu et al. (2016) [13]
ESR1	Hypomethylated	Lambertini et al. (2017) [25]
FERMT2	Hypermethylated	Makrinou et al. (2020) [30]
FL54034	Hypomethylated	Makrinou et al. (2020) [30]
GHRHR	Hypomethylated	Sagvekar et al. (2019) [28]
HAPLN1	Hypermethylated	Sagvekar et al. (2019) [28]
HOOK2	Hypermethylated	Jacobsen et al. (2019) [27]
IGF1BP2	Hypermethylated	Yu et al. (2015) [24]
INSR	Hypomethylated	Yu et al. (2015) [24]
	Hypomethylated * Hypermethylated**	Jones et al. (2015) [23]
IRS1	Hypermethylated	Kokosar et al. (2016) [15]
KLF10	Hypomethylated	Nilsson et al. (2018) [26]
LHCGR	Hypomethylated	Wang et al. (2014) [32] Jones et al. (2015) [23]
LIF	Hypermethylated	Sagvekar et al. (2019) [28]
LINE1	Hypermethylated	Pruksananonda et al. (2016) [34]
LPCAT1	Hypermethylated	Mao et al. (2021) [31]
LPL	Overexpressed	Leung et al. (2020) [22]
MAMLD1	Hypomethylated	Sagvekar et al. (2019) [28]
NAMPT	Hypomethylated	Nilsson et al. (2018) [26]
NCF2	Hypomethylated*	Xu et al. (2016) [13]
NR4A1	Hypomethylated	Pan et al. (2018) [33]
NRIP1	Hypermethylated	Yu et al. (2015) [24]
PARK2	Hypermethylated	Lambertini et al. (2017) [25]
PAX6	Hypermethylated	Lambertini et al. (2017) [25]
PCYT1A	Hypermethylated	Mao et al. (2021) [31]
PPARG	Hypermethylated	Kokosar et al. (2016) [15]
PPARGC1	Hypermethylated	Zhao et al. (2017) [35]
PTGER1	Hypermethylated	Sagvekar et al. (2019) [28]
RAB5B	Hypermethylated	Jones et al. (2015) [23] Kokosar et al. (2016) [15]
RBPMS	Hypomethylated	Makrinou et al. (2020) [30]
RETN	Hypomethylated	Sagvekar et al. (2019) [28]
SCGB1D4	Hypomethylated	Hiam et al. (2019) [35]
SCNA	Hypomethylated*	Xu et al. (2016) [13]
SFRP1	Underexpressed	Leung et al. (2020) [22]
SHH	Hypermethylated	Makrinou et al. (2020) [30]
SLC2A4	Overexpressed	Leung et al. (2020) [22]
SLC2A8	Hypermethylated	Yu et al. (2015) [24]
SVEP1	Hypermethylated	Kokosar et al. (2016) [15]
TGFB1	Hypomethylated	Lambertini et al. (2017) [25]
TNF	Hypermethylated	Sagvekar et al. (2019) [28]
WNT10B	Underexpressed	Leung et al. (2020) [22]
YWHAQ	Hypomethylated*	Xu et al. (2016) [13]

*Comparison between obese women with PCOS and non-obese women with PCOS, **Comparison of skeletal muscle tissue in obese women with PCOS and non-obese women with PCOS

Other studies have highlighted epigenetic alterations in the RAB5B, member RAS oncogene family (*RAB5B*), which encodes a member of the Ras-related GTPase superfamily responsible for the transport of intracellular vesicles and endosome formation related to diseases, such as obesity and type 1 diabetes mellitus [15, 23]. Jones et al. [23] reported that *RAB5B* was much less expressed in women with PCOS than in the control group of healthy women. Consistent with these results, Kokosar et al. [15] showed significantly lower mRNA expression of the *RAB5B* gene in the adipose tissue of women with PCOS compared to healthy women without PCOS.

Other genes that have undergone epigenetic alterations are *BNIP3*, *GHRHR*, and *TNF*, which have been correlated with the appearance of the clinical characteristics of hyperandrogenism [28, 33]. A study of *BNIP3* gene, responsible for participating in the metabolism of lipid precursors in the biosynthesis of androgens, showed that hypomethylation of this gene's promoter correlated with a higher expression of *BNIP3* in women with PCOS [33]. However, the discussion of the study contradicts the results presented. The authors state that a lower expression of *BNIP3*, related to gene hypermethylation instead of a higher expression, probably results in an excess of lipids, which contributes to hyperandrogenism [33]. Sagvekar et al. [28] observed hypomethylation and, therefore, an overexpression of *GHRHR*, responsible for regulating the release of somatotropin (GH) in the ovary, in granulosa cells of women with PCOS. According to the authors, this increased expression may be an indirect mediator of androgen excess in PCOS, as high levels of GH increase the sensitivity of developing ovarian follicles to gonadotropins [39]. Sagvekar et al. [28] also related the hypermethylation of the *TNF* gene, whose protein is responsible for suppressing the expression of *LHCGR* induced by FSH [40], with an indirect contribution of hyperandrogenism in PCOS [28].

In addition, Jiao et al. also reported the possibility that women with PCOS are more prone to developing cancer [41]. Patients with irregular menstruation and PCOS generally have hypomethylated global DNA in their ovarian

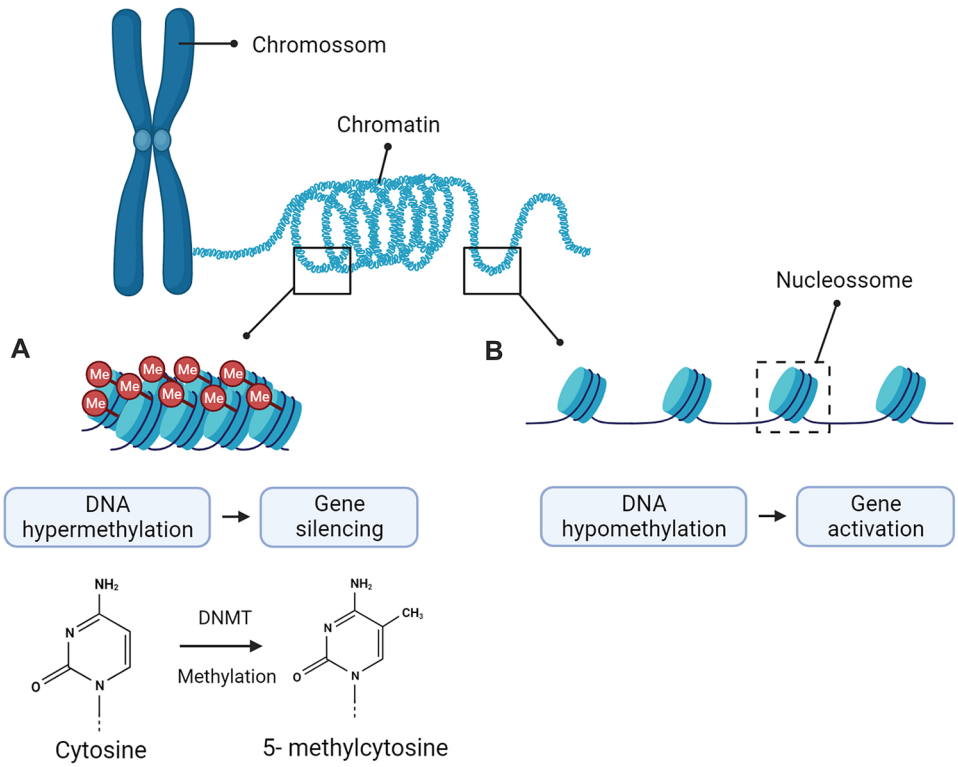

Fig. 3 DNA methylation. According to epigenetic modifications, regions of chromatin can be transcriptionally silenced (chromatin condensation) (**A**) or activated (chromatin decondensation) (**B**). The genes involved in PCOS present epigenetic modifications in DNA, leading to its hypermethylation (**A**) or hypomethylation (**B**), which are involved in gene silencing and activation, respectively. DNA methylation occurs through the conversion of cytosine to 5-methylcytosine through DNA methyltransferase (DNMT). Created by BioRender.com

tissues, a common feature in cancer tissues [41]. According to the authors, hormone levels in an irregular menstrual cycle are atypical, which may be a starting point for future studies that correlate cancer development in women with PCOS and hormonal changes. In the same study, the BRCA1 DNA repair associated (*BRCA1*) was altered in the ovarian tissues of women with PCOS at three local points (c154C, c1337G, and c2566T) [41]. It was not possible to relate these alterations to ovarian cancer, but possibly to the progression of breast cancer [41], which is an extremely relevant biomarker for future studies that have a relationship between the development of PCOS and breast cancer [41].

Upon reviewing the articles, it can be stated that epigenetic DNA methylation pathways affect the expression of the main genes involved in the etiology of PCOS. Among the various genes reported, *INSR*, *LHCGR*, and *RAB5B* were identified as fundamental to understanding the disease. The *INSR* and *LHCGR* genes were hypomethylated, while the *RAB5B* gene was hypermethylated in women with PCOS.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00404-023-07025-5.

Authors contributions All authors contributed to the study writing and data management. The study conception, design, and data collection were performed by AGM and LRF. The first draft of the manuscript was written by AGM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. AGM: data collection, management, and analysis, manuscript writing. MMS: data management and analysis; manuscript editing. LRF: data collection, management, and analysis, manuscript writing/editing.

Funding The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability All data relevant to the review are included in the article or uploaded as supplementary information.

Declarations

Conflict of interest The authors declare that there are no conflicts of interest with respect to the work reported in this article. The authors have no relevant financial or non-financial interests to disclose.

References

1. Fauser BCJM, Tarlatzis BC, Rebar RW et al (2012) Consensus on women's health aspects of polycystic ovary syndrome (PCOS): The Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril 97(1):28-38.e25. https://doi.org/10.1016/j.fertnstert.2011.09.024
2. Pfieffer ML (2019) Polycystic ovary syndrome: diagnosis and management. Nurse Pract 44(3):30–35. https://doi.org/10.1097/01.NPR.0000553398.50729.c0
3. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary

syndrome. Fertil Steril 81(1):19–25. https://doi.org/10.1016/j.fertnstert.2003.10.004
4. Wang J, Wu D, Guo H, Li M (2019) Hyperandrogenemia and insulin resistance: the chief culprit of polycystic ovary syndrome. Life Sci 236:116940. https://doi.org/10.1016/j.lfs.2019.116940
5. Ajmal N, Khan SZ, Shaikh R (2019) Polycystic ovary syndrome (PCOS) and genetic predisposition: a review article. Eur J Obstet Gynecol Reprod Biol X 3:100060. https://doi.org/10.1016/j.eurox.2019.100060
6. Haig D (2004) The (dual) origin of epigenetics. Cold Spring Harb Symp Quant Biol 69:67–70. https://doi.org/10.1101/sqb.2004.69.67
7. Vázquez-Martínez ER, Gómez-Viais YI, García-Gómez E, Reyes-Mayoral C, Reyes-Muñoz E, Camacho-Arroyo I, Cerbón M (2019) DNA methylation in the pathogenesis of polycystic ovary syndrome. Reproduction 158(1):R27–R40. https://doi.org/10.1530/rep-18-0449
8. Gibney ER, Nolan CM (2010) Epigenetics and gene expression. Heredity 105(1):4–13. https://doi.org/10.1038/hdy.2010.54
9. Jones PA (2012) Functions of DNA methylation: Islands, start sites, gene bodies and beyond. Nat Rev Genet 13(7):484–492. https://doi.org/10.1038/nrg3230
10. Kim JK, Samaranayake M, Pradhan S (2009) Epigenetic mechanisms in mammals. Cell Mol Life Sci 66(4):596–612. https://doi.org/10.1007/s00018-008-8432-4
11. Lawrence M, Daujat S, Schneider R (2016) Lateral thinking: How histone modifications regulate gene expression. Trends Genet 32(1):42–56. https://doi.org/10.1016/j.tig.2015.10.007
12. Peschansky VJ, Wahlestedt C (2014) Non-coding RNAs as direct and indirect modulators of epigenetic regulation. Epigenetics 9(1):3–12. https://doi.org/10.4161/epi.27473
13. Xu J, Bao X, Peng Z, Wang L, Du L, Niu W, Sun Y (2016) Comprehensive analysis of genome-wide DNA methylation across human polycystic ovary syndrome ovary granulosa cell. Oncotarget 7(19):27899–27909. https://doi.org/10.18632/oncotarget.8544
14. Cui P, Ma T, Tamadon A et al (2018) Hypothalamic DNA methylation in rats with dihydrotestosterone-induced polycystic ovary syndrome: effects of low-frequency electro-acupuncture. Exp Physiol 103(12):1618–1632. https://doi.org/10.1113/EP087163
15. Kokosar M, Benrick A, Perfilyev A et al (2016) Epigenetic and transcriptional alterations in human adipose tissue of polycystic ovary syndrome. Sci Rep [Internet] 6:22883. https://doi.org/10.1038/srep22883
16. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLOS Med 6(7):e1000097. https://doi.org/10.1371/journal.pmed.1000097
17. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P (2021) The Newcastle–Ottawa Scale (NOS) for assessing the quality if nonrandomized studies in meta-analyses. https://www.ohri.ca//programs/clinical_epidemiology/oxford.asp
18. Dasgupta S, Sirisha PVS, Neelaveni K, Anuradha K, Reddy AG, Thangaraj K, Reedy BM (2010) Androgen receptor cag repeat polymorphism and epigenetic influence among the south Indian women with polycystic ovary syndrome. PLoS ONE 5(8):e12401. https://doi.org/10.1371/journal.pone.0012401
19. Laisk T, Haller-Kikkatalo K, Laanpere M, Jakovlev U, Peters M, Karro H, Salumets A (2010) Androgen receptor epigenetic variations influence early follicular phase gonadotropin levels. Acta Obstet Gynecol Scand 89(12):1557–1563. https://doi.org/10.3109/00016349.2010.526182
20. Sang Q, Zhang S, Zou S et al (2013) Quantitative analysis of follistatin (FST) promoter methylation in peripheral blood of patients with polycystic ovary syndrome. Reprod Biomed Online 26(2):157–163. https://doi.org/10.1016/j.rbmo.2012.10.011
21. Saenz-de-Juano MD, Ivanova E, Romero S et al (2019) DNA methylation and mRNA expression of imprinted genes in blastocysts derived from an improved in vitro maturation method for oocytes from small antral follicles in polycystic ovary syndrome patients. Hum Reprod 34(9):1640–1649. https://doi.org/10.1093/humrep/dez121
22. Leung KL, Sanchita S, Pham CT et al (2020) Dynamic changes in chromatin accessibility, altered adipogenic gene expression, and total versus de novo fatty acid synthesis in subcutaneous adipose stem cells of normal-weight polycystic ovary syndrome (PCOS) women during adipogenesis: evidence of cellular programming. Clin Epigenetics 12(1):181. https://doi.org/10.1186/s13148-020-00970-x
23. Jones MR, Brower MA, Xu N, Cui J, Mengesha E, Chen YDI, Azziz R, Goodarzi MO (2015) Systems genetics reveals the functional context of PCOS loci and identifies genetic and molecular mechanisms of disease heterogeneity. PLOS Genet 11(8):e1005455. https://doi.org/10.1371/journal.pgen.1005455
24. Yu YY, Sun CX, Liu YK, Li Y, Wang L, Zhang W (2015) Genome-wide screen of ovary-specific DNA methylation in polycystic ovary syndrome. Fertil Steril 104(1):145–53.e6. https://doi.org/10.1016/j.fertnstert.2015.04.005
25. Lambertini L, Saul SR, Copperman AB, Hammerstad SS, Yi Z, Zhang W, Tomer Y, Kase N (2017) Intrauterine reprogramming of the polycystic ovary syndrome: evidence from a pilot study of cord blood global methylation analysis. Front Endocrinol 8:352. https://doi.org/10.3389/fendo.2017.00352
26. Nilsson E, Benrick A, Kokosar M et al (2018) Transcriptional and epigenetic changes influencing skeletal muscle metabolism in women with polycystic ovary syndrome. J Clin Endocrinol Metab 03(12):4465–4477. https://doi.org/10.1210/jc.2018-00935
27. Jacobsen VM, Li S, Wang A, Zhu D, Liu M, Thomassen M, Kruse T, Tan Q (2019) Epigenetic association analysis of clinical subphenotypes in patients with polycystic ovary syndrome (PCOS). Gynecol Endocrinol 35(8):691–694. https://doi.org/10.1080/09513590.2019.1576617
28. Sagvekar P, Kumar P, Mangoli V, Desai S, Mukherjee S (2019) DNA methylome profiling of granulosa cells reveals altered methylation in genes regulating vital ovarian functions in polycystic ovary syndrome. Clin Epigenet 11(1):61. https://doi.org/10.1186/s13148-019-0657-6
29. Echiburú B, Milagro F, Crisosto N et al (2020) DNA methylation in promoter regions of genes involved in the reproductive and metabolic function of children born to women with PCOS. Epigenetics 15(11):1178–1194. https://doi.org/10.1080/15592294.2020.1754674
30. Makrinou E, Drong AW, Christopoulos G et al (2020) Genome-wide methylation profiling in granulosa lutein cells of women with polycystic ovary syndrome (PCOS). Mol Cell Endocrinol 500:110611. https://doi.org/10.1016/j.mce.2019.110611
31. Mao Z, Li T, Zhao H, Wang X, Kang Y, Kang Y (2021) Methylome and transcriptome profiling revealed epigenetic silencing of *LPCAT1* and *PCYT1A* associated with lipidome alterations in polycystic ovary syndrome. J Cell Physiol 236(9):6362–6375. https://doi.org/10.1002/jcp.30309
32. Wang P, Zhao H, Li T et al (2014) Hypomethylation of the LH/choriogonadotropin receptor promoter region is a potential mechanism underlying susceptibility to polycystic ovary syndrome. Endocrinology 155(4):1445–1452. https://doi.org/10.1210/en.2013-1764
33. Pan JX, Tan YJ, Wang FF et al (2018) Aberrant expression and DNA methylation of lipid metabolism genes in PCOS: a new insight into its pathogenesis. Clin Epigenet 10(1):6. https://doi.org/10.1186/s13148-018-0442-y

34. Pruksananonda K, Wasinarom A, Sereepapong W, Sirayapiwat P, Rattanatanyong P, Mutirangura A (2016) Epigenetic modification of long interspersed elements-1 in cumulus cells of mature and immature oocytes from patients with polycystic ovary syndrome. Clin Exp Reprod Med 43(2):82–89. https://doi.org/10.5653/cerm.2016.43.282
35. Hiam D, Simar D, Laker R, Altıntaş A, Gibson-Helm M, Fletcher E, Moreno-Asso A, Trewin AJ, Barres R, Stepto NK (2019) Epigenetic reprogramming of immune cells in women with PCOS impact genes controlling reproductive function. J Clin Endocrinol Metab 104(12):6155-6170. https://doi.org/10.1210/jc.2019-01015
36. Wang XX, Wei JZ, Jiao J, Jiang SY, Yu DH, Li D (2014) Genome-wide DNA methylation and gene expression patterns provide insight into polycystic ovary syndrome development. Oncotarget 5(16):6603–6610. https://doi.org/10.18632/oncotarget.2224
37. Zhao H, Zhao Y, Ren Y, Li M, Li T, Li R, Yu Y, Qiao J (2017) Epigenetic regulation of an adverse metabolic phenotype in polycystic ovary syndrome: the impact of the leukocyte methylation of *PPARGC1A* promoter. Fertil Steril 107(2):467-474.e5. https://doi.org/10.1016/j.fertnstert.2016.10.039
38. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92(6):829–839. https://doi.org/10.1016/S0092-8674(00)81410-5
39. Bachelot A, Monget P, Imbert-Bolloré P, Coshigano K, Kopchick JJ, Kelly PA, Binart N (2002) Growth hormone is required for ovarian follicular growth. Endocrinology 143(10):4104–4112. https://doi.org/10.1210/en.2002-220087
40. Nakao K, Kishi H, Imai F, Suwa H, Hirakawa T, Minegishi T (2015) TNF-α suppressed FSH-induced LH receptor expression through transcriptional regulation in rat granulosa cells. Endocrinology 156(9):3192–3202. https://doi.org/10.1210/EN.2015-1238
41. Jiao J, Sagnelli M, Shi B et al (2019) Genetic and epigenetic characteristics in ovarian tissues from polycystic ovary syndrome patients with irregular menstruation resemble those of ovarian cancer. BMC Endocr Disord 19(1):30. https://doi.org/10.1186/s12902-019-0356-5