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Polycystic Ovary Syndrome

Functional Investigation and Clinical Application

Edited by Zhengchao Wang

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Meet the editor

Dr. Zhengchao Wang is a full professor at the College of Life Sciences, Fujian Normal University, China. He is a Principal Investigator for the General Program for the National Natural Science Foundation of China and the Program for New Century Excellent Talents at the University of the Ministry of Education of China. He is also a team leader in the Fujian Provincial Key Laboratory for Developmental Biology and Neurosciences, Key Laboratory of Optoelectronic Science and Technology for Medicine of the Ministry of Education. He obtained his Ph.D. in 2008 and has a long teaching and research career. He has published 172 scientific papers in English or Chinese, participated in approximately 32 grants, and published 12 books as the editor in his specialty and related diseases.

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Preface

Polycystic ovary syndrome (PCOS) is a major health problem. It is a heterogeneous hormone-imbalance disorder that occurs in reproductive-aged women worldwide and is characterized by hyperandrogenism, ovulatory process dysfunction and polycystic ovaries.

PCOS is affected by various factors and there are no unique diagnostic criteria in different regions due to the heterogeneity of its clinical manifestations and endocrine system changes. Therefore, it is often difficult to accurately diagnose women with PCOS, as the signs and symptoms of PCOS can vary among individuals. Although PCOS is usually diagnosed during the early reproductive years, its precise pathogenesis remains unclear. An increasing number of studies have demonstrated that the insulin signaling pathway has an important role in the pathophysiology of PCOS, including phosphatidylinositol 3-kinase and protein kinase B signaling, which is critically implicated in insulin resistance, androgen secretion, obesity and follicular development. PCOS manifests as defective ovarian steroid biosynthesis and hyperandrogenemia, and 50%–70% of women with PCOS exhibit insulin resistance and are hyperinsulinemic, indicating that insulin resistance and hyperinsulinism may have an important role in the pathophysiology of PCOS.

This book provides a comprehensive overview of the current state of the art in PCOS research to benefit the population of women with PCOS. It is my hope that this book is meaningful to the clinicians who care for women with PCOS and to the researchers who investigate the complexities of this disease. We sincerely thank all the authors for their contributions to our book. At last, we hope that this book is meaningful to the clinicians who care for women with PCOS and to the researchers who investigate the complexities of this disorder.

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Section 1

The Pathogenesis of Polycystic Ovarian Syndrome

Chapter 1

Pathophysiology of Polycystic Ovarian Syndrome

Manu, Thomson Soni, Victoria and Pranav Kumar Prabhakar

Abstract

Polycystic ovarian syndrome (PCOS) is the most common endocrinopathy that affects 8–20% of the reproductive age females and adolescent girls every year worldwide and approximately 5 million cases reported in the USA annually. It is more prevalent in urban areas as compared to the rural areas because of the difference in the lifestyles of rural and urban ladies. Rarely PCOS is passed on by heredity in some cases. It mostly occurs due to a lack of awareness. Its symptoms become mild to severe like initially hirsutism, acne which further leads to irregular periods and infertility. The pathogenesis of PCOS is not known because it is a complex multi-genetic disorder. Ovary and adrenal steroid genesis, the action of steroid hormone, action and regulation of gonadotropin, action, and secretion of insulin, obesity, and regulation of energy in PCOS involve genes. Its main clinical manifestations are insulin resistance and increased level of androgen. Metformin is used to sensitize the insulin because the risk of glucose intolerance also gets elevated with insulin resistance, type-2 diabetes, and lipid abnormalities. Likely, the outcome of different, deeply interrelated genetic abnormalities that influence each other and perpetuate the syndrome may be represented by PCOS.

Keywords: polycystic ovarian syndrome, insulin resistance, genetics of PCOS, metformin, gonadotropin

1. Introduction

Polycystic ovarian syndrome (PCOS), also known as hyperandrogenic anovulation (HA) or Stein-Leventhal Syndrome, is the most common endocrine disorder which affects reproductive age women [1, 2]. PCOS is a complex psychological, metabolic, and reproductive condition that is characterized by either hyperandrogenism or abnormal gonadotropin secretion and sometimes associated with insulin resistance [3, 4]. It refers to a disorder with a combination of reproductive [5], environmental and metabolic characteristics [6]. It also causes endometrial abnormalities such as fertility implication and cancer implication [7, 8]. It is most commonly found in the reproductive age group female [9, 10] but it can also affect males due to hormonal imbalances. The appearance of polycystic ovary under the transvaginal pelvic ultra-sound look are like small cyst. These cysts are eggs or follicles rimming the ovaries, which starts increasing in size and then stops at a tiny follicle size of around 2–10 nm.

They described infertile women with shinny ovaries, which is having multiple cysts in the size of approx. 2–10 mm. According to many pieces of research, PCOS may affect 8–15% women of reproductive age but 35% premenopausal mothers and 40% of sisters were affected by this problem as compared to general rates [11–13]. These ranges of incidence may vary according to the diagnostic criterion of the PCOS. In the case of PCOS, there are multiple cysts present in the woman's ovaries which are not released on its actual time so as a result immature follicle keeps growing, and leads to the formation of multiple cysts. There are reports which say 65–95% of all the women have PCOS also have insulin resistance which might be due to perturbed receptor tyrosine kinase, or other protein of insulin signaling cascade, modified adipokine signaling, and its secretion when compared to normal women [14, 15]. Increased level of insulin induces the rise in male sex hormone androgens, like testosterone which plays an important role in the pathogenesis [16, 17]. The exact pathogenesis of PCOS is still not very clear [18, 19]. There are several clinical significances like hirsutism, infertility, irregular periods, alopecia and many more symptoms begin shortly after puberty [20] and they develop during late terms and into early adulthood [3]. There is no particular treatment done to cure this problem but it can be managed by controlling sugar level and regulating the menstrual cycle by taking forming drug i.e. first insulin-sensitizing drug and it can also be treated by gonadotropin as first step treatment agents in ovulation. Low level of progesterone leads to cause overstimulation of immune system that produces the more estrogen and it will further lead to the production of autoantibodies i.e. anti-thyroid, anti spermatic, antinuclear, anti-ovarian, etc. there is a study in which we come to know that there are many proteins involved in PCOS [21]. The cumulative effect of modified protein, which are the product of mutated genes, along with various other factors like genetic inheritance and environment leads to complications in the case of PCOS [11]. Many genes participated in etiology of this syndrome but this is not fully investigated yet but the study shows that abnormality of genes in case of PCOS mostly affects the pathways of signal transduction which controls the steroidogenesis (formation of steroids) [12], insulin action [22] and secretion, gonadotropin action and regulation [23, 24], steroid hormones action and many more [25, 26].

2. Symptoms

There are many symptoms which are contributed to PCOS such as hirsutism, acne, alopecia, acanthosis, seborrhea, infertility, insomnia, and irregular periods (**Figure 1**) [27, 28].

Hirsutism: It is excessive growth of hair on a woman's face and body. In this case, there is a condition of unwanted hair growth in women mainly on the face, chest, and back, just as males [29].

Acne: It is a chronic skin condition that causes the spots and pimples. It mainly occurs when oil and dead skin cells clog the hair follicles which leads to the formation of whiteheads, blackheads, pimples, cysts, etc. They mainly occur on the face, shoulders, back, neck, chest, upper arms. It may also occur due to the different peripheral sensitivity of the androgen receptors [30].

Alopecia: It is the condition in which there is sudden hair loss which leads to baldness and in this condition, there is also thinning of hair.

Acanthosis: It is skin condition when there are dark velvety patches in the body folds and body creases like underarms and neck. The affected skin can become thicken and blackened.

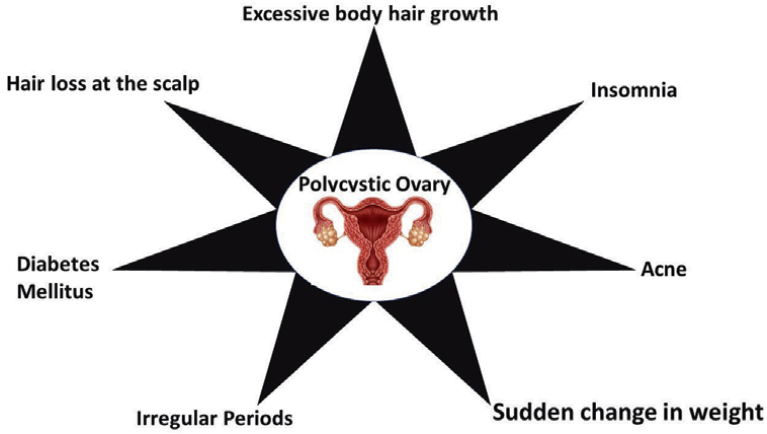

Figure 1.
Common symptoms of polycystic ovary syndrome (PCOS).

Seborrhea: It is a condition when there are patches and red skin mainly on the scalp. There may be yellow plaques on the scalp. It is also a chronic inflammatory disease.

Striae: This is also known as stretch marks. They appear as reddened streaks on the skin it is mainly due to rapid change in body weight or in case of pregnancy also.

Acrochordons: They also knew as skin tags. This is the common skin growth, which sticks out. They are small soft common benign.

Infertility: It is an inability to conceive after a long period with unprotected sex.

Insomnia or sleeping disorder: Women with PCOS reports for the poor sleep or insomnia. There are a number of factor which leads to poor sleep but the PCOS is associated with the sleep disorder called sleep apnea. In the case of sleep apnea person, stops breathing for some duration during sleep.

Irregular periods: It is a problem with menstrual cycles. It is a condition when there are delayed, missed, or more bleeding patterns [31]. It further leads to the problem in the reproductive system. With PCOS, there is a correlation to a low level of androgen with advancing age in women [32].

3. Causes

Exact etiology remains unknown but some of them are written below [33–35].

a. *High level of insulin:* Insulin is a polypeptide hormone, which is synthesized and released by pancreatic beta cells and its main function is to reduce the blood glucose sugar level in the body, which indicates that PCOS has metabolic and reproductive morbidity [36]. If there is no insulin production then there is a high level of sugar in the body. It will act as a driving force for hyperandrogenism [37]. There is insulin resistance also occurring in which insulin is produced by the pancreas but our body not able to use that insulin [38]. A high level of insulin induces the ovaries to produce more androgens such as testosterone which will prevent ovulation [39, 40]. In PCOS pregnancies, unable to prevent excess testosterone [41]. There are two possibilities in the case of hyperinsulinemia i.e. increased hyperandrogenemia [42, 43] and decreased the circulating level of sex hormone-binding globulin [44]. Peripheral insulin resistance is also related to uterine and ovarian problems [45].

b. *Bad dietary choices:* Eating junk food cause PCOS because junk food contains excess fat, simple carbohydrates, and sugar which leads to a high risk of obesity [46], Diabetes, cardiovascular disease, which further leads to PCOS.

c. *Weak immune system:* It is the common cause of menstrual irregularities because a low level of progesterone in PCOS causes overstimulation of the immune system that produces more estrogen which leads to producing autoantibodies.

d. *Obesity:* Obesity is the major cause of PCOS because when we eat more junk food, which causes obesity and an obese person more prone to get diabetes which ultimately leads to PCOS [47]. Insulin resistance and high level of insulin in the blood, which further stimulate ovarian androgen production are associated with obesity [47, 48]. Similarly, the prevalence of dyslipidemia also rises with increasing obesity [49, 50]. Obesity also causes a high risk for several cancers like breast cancer, endometrial cancers, etc. [8, 51–53].

e. *Inflammation and oxidative stress:* Inflammation is considered as one of the key features of endothelial dysfunction and atherosclerosis. A lady with PCOS is prone to have a high level of visceral adiposity in all categories of BMI. This high level of visceral adipocytes is linked with insulin resistance, a rise in blood glucose, and lipid levels [54]. These adipocytes affect endocrine as well as exocrine. Inflammation and oxidative stress are very closely interrelated. The inflammatory process generates reactive oxygen species and oxidative stress process and products induce and aggravates inflammation. There are literature reports available that say the lipid peroxidation level increased in the case of PCOS and this rise is having a positive correlation with the BMI, insulin level, and blood pressure [55]. Women having PCOS also have a decreased number of antioxidants, glutathione, as well as haptoglobin. In these cases, the susceptibility towards the oxidative stress-induced DNA damage also increases. Oxidative stress also involved in many abnormalities od the reproductive system as well such as infertility, endometriosis, anovulation, and defects in the quality of oocyte [54, 55].

f. *The genetic tendency for PCOS:* There are many genes, which are responsible to cause PCOS. It may be occurred among the population and within the families. The critical genetic variations in PCOS across different ethnicities and their associated effects such as hyperandrogenism in women [56], insulin resistance [3, 17, 22, 56–58], miscarriage, recurrent pregnancy loss, endometrial receptivity [59]. Women suffering from PCOS are more prone to different types of cancers [8, 53, 60, 61].

4. The difference in normal ovary and PCOS ovary

In normal case ovaries, fallopian tubes, uterus, vagina are the main reproductive organs of females and they are having a lifetime supply of ovum and these ova are stored in sac-like fluid-filled structures called follicles. The sex hormones, which are helpful to act on the function of the ovaries, are produced by the pituitary gland located just below the hypothalamus at the base of the brain. So pituitary gland secretes follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the bloodstream. Through blood, these hormones reach the ovary where they start to get maturing the immature ovum and increases the size of follicles [62]. As the eggs get

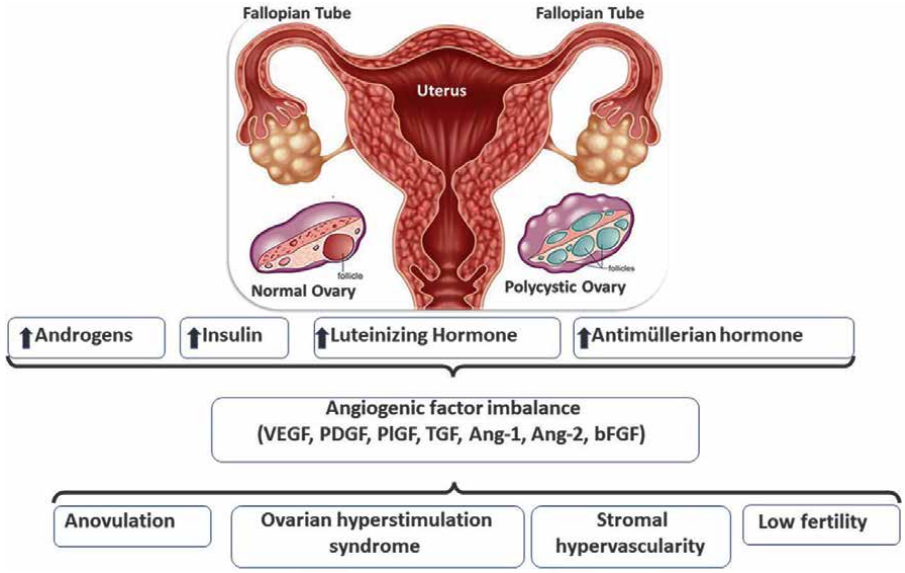

Figure 2.
Pathophysiology of polycystic ovary syndrome.

matured the follicles start releasing estrogen, a female sex hormone. As soon as the estrogen level crosses threshold concentration, the pituitary gland senses the LH flow to ovaries and results in the rapturing of the mature follicles and releases its ovum/egg. This process is known as ovulation. The free ovum then passes through the fallopian tube where the fertilization process occurs and the remaining immature follicle gets dissolved. If ovum does not get fertilized then the egg and line of the uterus are shared to doing the next menstrual cycle. But in the case of PCOS, the Pituitary gland secretes a higher amount of LH due to biochemical destruction which disturbs the menstrual cycle. Then there are no mature follicles present so no ovulation occurs and it will lead to infertility. Some follicles do not dissolve, they remain there as such and formed as fluid-filled sac-like structures which are known as CYSTS (**Figure 2**). Increased level of insulin and LH will produce testosterone [62, 63] which causes hirsutism, acne, prevent ovulation which further leads to infertility [64].

5. Genetics of PCOS

PCOS is a complex genetic disease with several susceptibility genes. It has powerful genetic and environmental components [65]. Many pieces of research show that identical twins are more prone to get PCOS than fraternal twins or non-twin siblings. Women having a 50% chance to get PCOS if their mother or sibling also has this disease. Research also shows that the male siblings of women with PCOS are more susceptible to get insulin resistance than the male sibling of unaffected women. Genes that are linked with PCOS are responsible for the production and metabolism of sex hormones or linked with an impaired function of insulin. Genes involved in PCOS are

a. *DENND1A* gene which is linked to PCOS risk and this gene is responsible in the import of molecules into the cell which is responsible for the recycling of hormone receptors from the cell's surface and leads to PCOS,

b. *THADA* gene is linked with type-2 diabetes mellitus [66] and some other cancers and the production of this gene in the pancreas by releasing some hormones which regulate the blood sugar levels in the body,

c. *SHBG* gene is sex hormone-binding globulin is a biomarker of PCOS and if there are low levels of SHBG then more hormones free for biological action in the body and then they promotes the symptoms of PCOS,

d. *FBN3* gene is placed on 19 chromosomes within the gene and it is linked with PCOS and insulin resistance women who are already having PCOS and if there is the lower level of FBN3 in PCOS affected women then they lead to abnormally increase level of TGF-beta activity which causes many metabolic disorders like hypertension, hyperlipidemia [67, 68], inflammation and cardiovascular diseases (CVD).

e. *LHCGR* is luteinizing hormone/chorionic gonadotropin receptor are the genes which control the development of sex organs and hormones in males but in the case of females, they are responsible for ovulation.

f. *INSR* gene is an insulin receptor gene that encodes the receptor for the hormone insulin etc. [57]. These receptors are extracellular receptors present on the plasma membrane having three different regions like extracellular, transmembrane, and intracellular. Extracellular parts of the receptor receive insulin while the intracellular portion is tyrosine kinase in nature and gets phosphorylated which finally amplifies the signal inside the cells for various physiological roles.

5.1 Genes which are responsible for Ovary and adrenal steroidogenesis

a. *CYP11A:* The initial step of steroidogenesis is the conversion of cholesterol into progesterone which is catalyzed by P450. Then the gene CYP11A which is located at 15q24 encodes P450. The association in the level of serum testosterone has been shown by this gene [69, 70]. The CYP11A alleles also show the association with 5'UTR.

b. *CYP17A1:* This CYP17A1 gene help to convert pregnenolone and progesterone into 17-hydroxypregnenolone and 17-hydroxyprogesterone and also help to invert dehydroepiandrosterone and Δ4-Androstenedione which is created by an enzyme i.e. P450c17. Activities of this enzyme like 17,20-lyase and 17-hydroxylase. P450c17 is encoded by CYP17A, and this gene is located at 10q27.3. It was proclaimed that the women having PCOS has increased activity and expression in ovary theca cells.

c. *CYP19:* The gene CYP19 helps to converts the androgen into estrogen. This enzyme is made up of cytochrome P450 aromatase and NADPH cytochrome P450 reductase. P450 aromatase is encoded by gene i.e. CYP19 which is located at 15p 21.1. Deficiency of aromatase enzyme mostly occur in those people whose is having high androgen level and this aromatase activity is decreased in follicles because as compared to normal follicles PCOS follicles having estradiol which lowers the aromatase stimulation bioactivity and the excess level of androgens leads to improper development of follicles [66].

d. *HSD17B1 & HSD17B2:* The group of alcohol oxidoreductase includes these genes, which is used to catalase the dehydrogenation of 17-hydroxysteroids in the steroidogenesis process. There is an interconversion of androstenedione and testosterone, DHEA and androstenediol and estrone, and estradiol. The level of expression of mRNA synthesizing and inactivating enzyme has been reported high in women without the PCOS endometrial treatment [71].

e. *HSD3B1 & HSD3B2:* The placenta and peripheral tissues show Type I 3β-HSDiso-enzyme but the adrenal gland, ovary, and testis show type II 3β-HSDiso-enzyme. The deficiency of HSD3B in hyperandrogenic women is related to the insulin-resistant PCOS.

f. *StAR:* StAR is a Steroidogenic Acute Regulatory protein. The cholesterol within the mitochondria is transported by such kind of transport protein. But in some patients, defect of steroidogenesis causes PCOS, due to which level of the ovary and adrenal androgen production rises. The transport protein i.e. StAR initiates the process of steroidogenesis [72].

6. Role of hormones in the PCOS

6.1 Steroid hormone actions

PCOS is one of the most common endocrine disorders in females of reproductive age group with multiple manifestations. The reproductive physiology of female is highly affected by her body weight and the metabolic status of her body. PCOS is mainly linked with obesity with the deposition of fats in abdominal regions in almost 51% of women having PCOS. As it is associated with insulin resistance and hence results in hyperlipidemia, cardiovascular disorders, and also cancer of the endometrium. There are some important components of lipid metabolic pathways which play a significant role in the PCOS occurrence.

a. *Androgen receptor:* PCOS is most commonly characterized by more secretion of androgen. This will increase the level of androgen production by the ovaries i.e. hyperandrogenism which is the second most common characteristic in PCOS. 17–83% of women are in occurrence to PCOS [73].

b. *Serum Sex hormone-binding Globulin (SHBG):* SHBG, a glycoprotein, influences the bioavailability of lipid-soluble steroid hormones. The serum SHBG level has been reported in patients with hyperandrogenism and PCOS. It results in hyperinsulinemia [43, 45], which will lead to lower the level of SHBG. And it will also suppress the SHBG synthesis in the liver [74].

6.2 Gonadotropin action and regulation

Kisspeptin is a protein that is coded by the KISS1 gene. Initially, this protein was discovered as its role in the tumor suppression mainly for melanoma and breast cancers. Kisspeptins have recently emerged as essential upstream regulators of GnRH neurons with many roles in reproduction such as puberty onset [75, 76], brain sex differentiation [77], gonadotropin secretion [78], ovulation and metabolic regulation of fertility [79].

i. *Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH):* PCOS is characterized by an inappropriate gonadotropin secretion. One of the common reasons for PCOS is an elevated level of LH. High LH secretion and low FSH secretion has been seen in females affected with PCOS. The ratio used to indicate abnormal gonadotropin secretion is normally 2–3/1. The decreased level of FSH which stimulates the growth of follicles in ovaries is a characteristic feature of PCOS. They are having mature eggs. The absence of FSH for a longer period will be responsible for immature follicles and eggs will not be released. Thus, this would result in infertility. Thus, small cysts will be produced in ovaries due to immature follicles [80, 81].

ii. *Inhibin βA and Inhibin βB:* Insulin resistance is highly associated with PCOS. FSH secretion is regulated by a heterodimer, Inhibin. The release of inhibin suppresses the increase in FSH concentration. It has got two variants Inhibin A and Inhibin B which are secreted by gonads, pituitary gland, placenta, etc. During the follicular phase, Inhibin B is more important than Inhibin A. The level of inhibin is higher in a woman with PCOS than the normal ones.

iii. *MADH4:* Mothers against decapentaplegic homolog 4 are a protein involved in signaling in mammals. The protein belongs to the SAMD family. SAMD4 has two functional domains MH1 and MH2 consist of a tridimensional structure (Regions M and H represent MAD homology). This resembles a similarity between SAMD4 in mammals and Drosophila protein.

6.3 Insulin action and secretion

Many women having PCOS have shown glucose-induced hyperinsulinemia, insulin resistant, independent body mass index. The insulin-dependent glucose level decreases by 35–40% in the case of PCOS affected women when compared to normal women. More than 2% of women with PCOS moves from normal to type 2 diabetes mellitus and almost 16% of women move from impaired glucose tolerance to type 2 diabetes mellitus. The incidence of PCOS with obesity is very complex. Although PCOS occurs both in obese and lean women some recent studies and meta-analysis reveal that obesity more frequently occurs in women with PCOS. And it is a well-known fact that obesity leads to insulin resistance and finally to diabetes mellitus type 2. To fulfill the body's requirement, the pancreas produces a high amount of insulin and a condition of hyperinsulinemia occurs. This condition mainly affects fibroblasts and adipocytes. One of the main effects of this hyperinsulinemia is the autophosphorylation of tyrosine in the insulin receptor decreases whereas the autophosphorylation of serine increases in both types of cells. In the fibroblast, the insulin-dependent glucose uptake, translocation of GLUT4 to the plasma membrane, and insulin-dependent glycogen synthesis decrease whereas in the case of adipocytes also glycogen synthesis decreases. Insulin influences the function of LH on to the ovary which increases the production of androgens. Insulin also inhibits sex hormone-binding globulin (SHBG) production by hepatocytes increases the free androgen fraction in blood circulation. An increase in adipocyte tissue also increases the severity of insulin resistance. Hence, it exacerbates the metabolic and endocrine derangements of PCOS (**Figure 3**).

i. *Insulin and IGF-I:* Growth of ovary is stimulated by insulin and IGF-I. The action of gonadotropins on ovary steroid synthesis is increased by them.

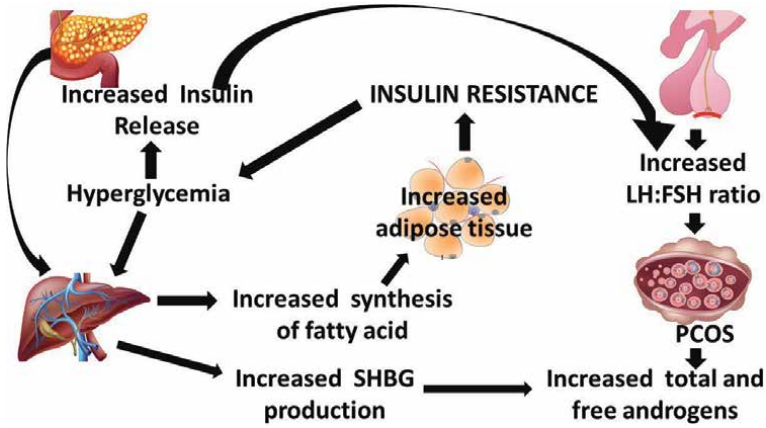

Figure 3.
Role of insulin in polycystic ovary syndrome.

The concentration of IGF-I and androgens is augmented by Insulin. It does this by regulating the synthesis of IGFBP-1 and SHBG in the liver. One of the common symptoms of PCOS is resistance to insulin. The important reasons resulting in PCOS is increased in insulin level and IGFBP-1 activity.

ii. *Insulin VNTR gene, IGF-II, insulin receptor substrate, and insulin receptor gene:* The insulin gene consists of a changing number of tandem repeats present at the 5'regulatory region. The main reason for the regulation of the translational rate of insulin is Polymorphism of the VNTR gene. It is also responsible for the regulation of gene encoding IGF-II. Class-I alleles are made up of a length of 40 tandem repeats and Class-II alleles are made up of 80 tandem repeats. Insulin resistance caused by PCOS may directly affect the pancreatic beta-cell. Insulin resistance in some PCOS phenotypes is affected by VNTR polymorphism. SNP at the tyrosine domain of the insulin receptor is greatly found to be associated with PCOS.

iii. *Peroxisome proliferator-activated receptor gamma (PPAR-γ):* It is a nuclear transcription factor that is involved in the regulation of glucose, lipid metabolism, and ovary steroidogenesis. Proline and Alanine in exon B are the most extensive findings on polymorphism in PPARγ. C1431T in exon6 is another polymorphism studied in the PPARγ gene. This variation is associated with PCOS. Insulin resistance path physiology is influenced by the PPARγ gene in women affected with PCOS.

6.4 Obesity and energy regulation

i. *Leptin and leptin receptor:* Leptin plays a major role in the pathological process of PCOS. Leptin and the free leptin index levels are higher in PCOS affected obese females than in thin PCOS females. In the case of PCOS, there is a high level of free leptin index but the low level in leptin receptors. And these both factors in PCOS, dependent on Body Mass Index (BMI).

ii. *Pro-opiomelanocortin (POMC):* It is a 16 K fragment that is used for the identification of factors that are responsible for excessive adrenal androgen levels.

iii. *UCP2 + 3:* Androgen synthesis of granulosa cells of affected PCOS patients is controlled by UCP2 which is an uncoupling protein. Treatment with the T3 hormone increases the expression of ovary UCP2. It may also change the pregnenolone synthesis which further results in P450 sec expression. This will further affect testosterone production.

7. Diagnosis

Diagnosis is the main purpose to detect or to identify the disease by seeing their symptoms, or by performing many tests. Like in the case of PCOS doctors may see the sign and symptoms and may also do to test for PCOS [82].

Appearance: Diagnosis of PCOS occurs by seeing the appearance of ovaries like in case of PCOS there are polycystic ovaries due to having more than 12 follicles present in it which cause enlargement of the ovary.

Medical history: To diagnose PCOS doctors may check a patient's medical history like is there any person already having the same problem in the patient family.

Symptoms: The doctor may check all the signs and symptoms of that disease, for example, hirsutism, acne, alopecia, acanthosis, seborrhea, striae, acrochordons, infertility, fatigue, pelvic pain, mood changes, sleeping problems, irregular periods. The person with PCOS is more prone to mental health problems like depression, anxiety because it is a chronic disease with increase male hormone i.e. testosterone causing problems and this hormone during pregnancy having reported increasing the risk of neurodevelopmental disorders. They may also have hypertension, high cholesterol, heart attack, sleep problem, diabetes, and breast cancer.

The Rotterdam criteria for the diagnosis of PCOS: A group of scientific experts, in 2003, elaborated the diagnostic criteria to include the ultrasound images of polycystic ovaries as another diagnostic marker and if two out of three diagnostic criteria will were met and the same endocrinopathies were excluded (**Figure 4**). This is known as

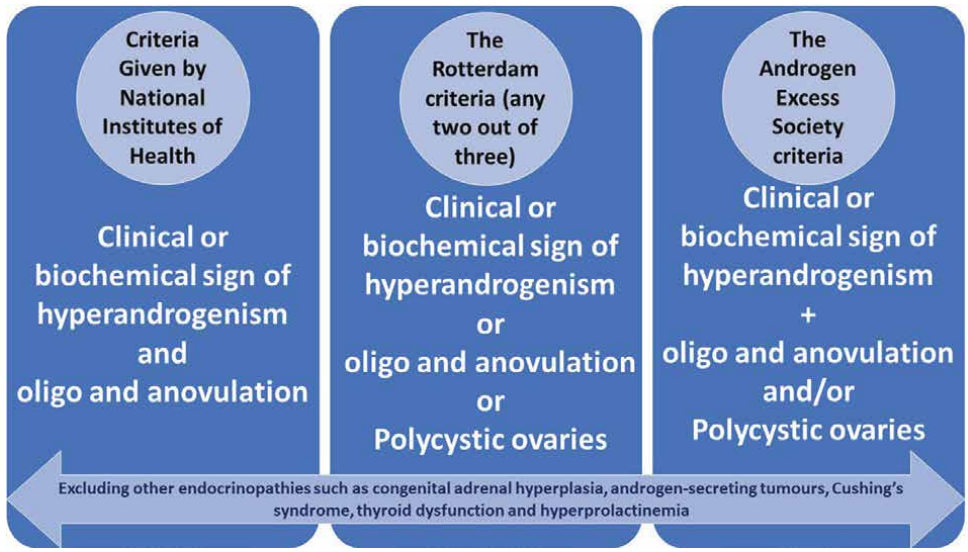

Figure 4.
The Rotterdam criteria for polycystic ovary syndrome.

Rotterdam criteria [83]. Slowly and steadily these criterions were accepted by various societies and commitees like European Society for Human Reproduction and Embryology (ESHRE), and the American Society for Reproductive Medicine (ASRM). Although this criteria is controversial and the Androgen Excess Society (AES) come up with a new set of diagnostic criteria in 2006 which are still the most commonly adopted criteria by different guidelines [84]. These guidelines are accepted and used by a wide group of obstetricians and gynecologists as well as other specialists.

Blood test: There are many tests done to check or to access the PCOS:

i. *Hormonal blood test:* These tests are used to check the level of hormone in our body. The most important hormonal test to check whether women may have hyperandrogenism is tested for androgens like testosterone and free androgen index. There are many other tests performed to detect the hormonal level, which may affect mensuration and ovulation. These tests include LH, FSH, Estrogen, sex hormone-binding globulin, dehydroepiandrosterone sulfate, androstenedione, thyroid-stimulating hormone, prolactin, hormones related adrenal function test example: 17-hydroxyprogesterone.

ii. *Another blood test:* As in PCOS; insulin resistance and weight are the main causes; therefore, the risk of cardiovascular disease and diabetes is necessary to be assessed. There are many tests perform to check these conditions. These tests include:

- Cholesterol
- Blood pressure
- Diabetes
- Glucose tolerance test etc.

Ultrasound: It is a type of imaging that is used to look at organs and structures inside the body. To identify any cyst, which is present in ovaries, and to check the size of ovaries whether they are enlarged or small, ultrasound of uterus, ovaries, and pelvis is suggested. Transvaginal ultrasound is a painless test with no radiations, it is performed on sexually active women otherwise abdominal scan can be done to check where is the ovaries are viewed from the outside through the stomach walls. In this type of ultrasound, a pen-shaped probe with an ultrasound sensor on the tip of the probe is used. This is helpful to see the clearer picture than an abdominal ultrasound.

8. Treatment

Unfortunately, PCOS cannot be cured, it can only manage by controlling the symptoms by doing exercise or by taking a healthy diet. It can be managed best to regulate their menstrual cycle and lower blood glucose level [65]. High fiber food like broccoli, cauliflower, sprouts, green and red pepper, olive oil, almonds, spinach, walnuts, fruits, etc. may help to reduce the impact of sugar in the body. Women with PCOS are majorly suffering from infertility so fertility drugs are given to aid anovulation [85]. Women with PCOS having hirsutism and acne problems are recommended to complete the

course of anti-androgen and do exercise for 45 minutes daily. Metformin is prescribed to lower the insulin level and it also aids in the regulation of the menstrual cycle and improves ovulation and pregnancy rates [86]. Metformin which is used for the treatment of diabetes for a long time is only a remaining member of the biguanide family. It will also help by improving the sensitivity of peripheral tissue against insulin [87]. It also inhibits hepatic gluconeogenesis [88, 89]. It also helps to reduce fatty acid oxidation. The dose of metformin is 500 to 2500 per day, an increase of dose may lead to worsening of side effects. Spironolactone is a steroid that acts as an antiandrogen, is chemically related to mineralocorticoid aldosterone. It blocks the synthesis of androgen to a particular extent [89, 90]. So, it is being used for the treatment of anovulation [91] and hyperandrogenism mainly for hirsutism [91, 92]. This drug in PCOS is limited [93], it has a good impact if used in a limited amount [94, 95]. One of the major factors for PCOS is the reduction of antioxidants and a rise in oxidative stress. Diabetes mellitus also leads to oxidative stress due to hyperglycemia. The supplementation with antioxidants has shown a positive result in the severity of diabetes alone and also improved insulin sensitivity in the case of PCOS. The intake of antioxidant or antioxidant containing food can be a good strategy to manage PCOS [96].

9. Significance of metformin in PCOS management

Metformin is a biguanide having the action for the reduction of glucose levels by increasing its utilization and also lowers down the androgen levels [97, 98]. The first insulin-sensitizing drug [99, 100] used to check the role of insulin resistance in PCOS is Metformin [101]. But according to research, Metformin alone is not a first-line treatment for the management of PCOS [102, 103]. Normal dosage for Metformin is 500–2500 mg/day [104]. According to research, a particular period of metformin dose of 1500 mg/day leads to a huge decline in the levels of circulating androgens and BMI [33]. It will help to regulate and improve the menstrual and help in reduction in circulating androgens levels and it will also help in reduction in body weight [105]. Thiazolidinediones are also used in the management of PCOS [106, 107]. Because it may improve the menstrual cycle and also help to reduce the androgen levels but with the help of this, there is no change in body weight. Metformin affects ovarian steroidogenesis [108]. The addition of metformin to IVF will increase the pregnancy outcome and also help to decrease the risk of ovarian hyperstimulation syndrome. It improves the oocyte quality in PCOS patients undergoing IVF [109]. Metformin also used to reduce the BMI because taking metformin with a low-calorie diet will reduce the fat. Metformin reduces the hyperandrogenism by effecting on ovaries and adrenal gland [110], which further leads to, suppresses their androgen levels and reduce the LH and increase the sex hormone-binding globulin.

Metformin i.e. Fortamet, Glucophage, etc. by taking Spironolactone will lower the level of sex hormone but it can cause birth defects. So, do not take it during pregnancy or if any plan to get pregnant [111]. Orlistat stops the body from digesting some fat in your food so improve your cholesterol level that's why it may take to get weight loss. In the case of fertility, Clomiphene encourages steps in the process that triggers ovulation [86, 107]. Hypothalamus secretes a gonadotropin-releasing hormone [32], which binds its receptor on secretory cells of the adenohypophysis [112]. As a result of GnRH, gonadotroph produces LH and FSH, which help to regulate development growth menstruation and reproduction of the body [113].

10. Infertility treatment

To start treatment in a stepwise fashion from least aggressive to more aggressive treatment, the use of clomiphene citrate and IVF protocol [32, 82].

Step one: Clomiphene treatment: The main indication is irregular or absent ovulation. PCOS patient is an excellent candidate for the use of clomiphene but almost 50% of the patient experiences the failure of clomiphene. Clomiphene citrate is the effective method of inducing ovulation and improving fertility and its adverse effects are multiple pregnancies and ovarian cyst. This resistance and failure in ovulation induction with clomiphene citrate are also thought to be related to chronic low-grade inflammation [114].

Step second: Gonadotropin treatment: Gonadotropins are the natural next step for ovulation induction. One characteristic of ovulation induction in PCOS patients is the slow response and the risk for ovarian hyperstimulation syndrome and cyst formation. The most used current step up is characterized by a low starting dose, which is maintained for a longer period then increased only of the small amount per week. This protocol is associated with a low incidence of severe OHSS and multiple pregnancies.

Step third: IVF: The goal of induction of ovulation is the development of one or few ovulatory follicles and the goal of stimulation in In vitro fertilization (IVF) cycles is to obtain multiple follicles but without in occurring in ovarian hyperstimulation syndrome [112, 113].

11. Conclusion

The polycystic ovarian syndrome is a common endocrinopathy, which is characterized by hyperandrogenism, insulin resistance, and abnormal gonadotropin secretions. PCOS disturbs both reproductive and metabolic functions. Their symptom varies from mild to severe like hirsutism, acne, alopecia, striae, irregular periods, and ultimately leads to infertility. PCOS is a complex multi genetic disorder so its pathogenesis is unknown. Stein and Leventhal discover it in 1935. They described infertile women with shinny ovaries, which is having multiple cysts in the size of pigeon eggs. High insulin resistance, bad dietary choices, weakened the immune system and many more are main factors that promote the PCOS. It cannot be diagnosed only based on symptoms, so blood tests are done to measure hormonal levels, ultrasound also is done to check the reproductive organs, and personal and family history is also useful for diagnosis purposes. Some hormonal levels are measured when considered to PCOS i.e. LH, FSH, DHEAS, Prolactin, testosterone, Progesterone, Androstenedione. Many proteins are involved in PCOS. It is a familial condition so genes play a major role in PCOS. Genes who are linked with PCOS are responsible for the production and metabolism of sex hormones or linked with an impaired insulin function. Genes involved in PCOS are DENND1A, SHBG, THADA, FBN3, LHCGR, and INSR, etc. Oral contraceptives are used to reduce the androgen and LH levels with improvement in hirsutism, acne, body weight, and also help to regulate the menstrual cycle. Metformin is the most effective insulin-sensitizing drug. Treatment for infertility includes clomiphene, laparoscopic ovarian drilling, and gonadotropins.

Authors' contributions

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Chapter 2

Thyroid Dysfunction: In Connection with PCOS

Mariya Anwaar and Qaiser Jabeen

Abstract

As the prevalence of endocrine dysfunction is increasing and is associated with many complications including polycystic ovary syndrome (PCOS) which, itself is a risk factor of thyroid dysfunction. Although the causality of this association is uncertain, the two conditions share a bidirectional relationship. Both syndromes share certain common characteristics, risk factors and pathophysiological abnormalities, which can be managed by lifestyle changes as well as pharmacological treatment. Polycystic appearing ovaries are a clinical feature of hypothyroidism as well as hyperthyroidism in a few case studies. Adiposity, evidence of deranged autoimmunity, increased insulin resistance and disturbed leptin levels are present in both the disease states, seeming to play a complex role in connecting these two disorders. Major endocrine pathways including hypothalamic-pituitary-thyroid axis (HPTA) and HP-gonadal axis are involved in parallel relationship of PCOS and thyroid dysfunction. This chapter helps to explore all the dimensions of the relationship between PCOS and thyroid dysfunction.

Keywords: thyroid dysfunction, hypothyroidism, hyperthyroidism, PCOS, HPTA

1. Introduction

Thyroid dysfunction as well as polycystic ovary syndrome (PCOS) are very common endocrine disorders among the general population. Although, thyroid dysfunction and PCOS have completely different etiopathogenesis, but have various common features. In primary hypothyroidism, an increased ovarian volume and cystic changes in ovaries have been reported. It is also increasingly recognized that thyroid dysfunction is more common in females with PCOS as compared to the healthy individuals [1, 2]. This is may be due to some common considerations as well as pathophysiological connection between PCOS and thyroid disorders leading an individual towards both the disorders. Considering the high prevalence of Hashimoto's thyroiditis (HT) and the high prevalence of PCOS in women in the reproductive period, the emphasis will lie on the possible etiological and clinical connections between HT and PCOS.

2. Endocrine system

The endocrine system is a network of glands that produce and secrete hormones to regulate many physiological processes [3]. The endocrine system is comprised of

hypothalamus, pituitary gland, pancreas, adrenal gland, ovaries, testes, pineal gland, thyroid gland, parathyroid gland and thymus gland [4]. These glands communicate with each other through different pathways called axis. Major endocrine pathways include hypothalamic-pituitary-thyroid axis (HPTA), hypothalamic-pituitary-gonadal axis, hypothalamic-pituitary-adrenal-axis, renin-angiotensin-aldosterone axis and hypothalamic-pituitary-adipose axis [5]. Endocrine glands are also closely linked with stress system, gut microbial flora and immune system [6].

2.1 Endocrine feedback system

Hormones are required for maintaining homeostasis and optimum body functions. Adequate secretion of hormones is ensured through biological feedback system that aims to provide hormones in a specific physiological range. Feedback system, is combination of several axis, that regulates endocrine and neural responses after any external or internal stimuli [7]. There are two types of feedback systems; positive feedback mechanism and negative feedback mechanism. Thyroid hormones exert both positive and negative feedback mechanism, which controls the release of both thyrotropin-releasing hormone (TRH) from hypothalamus and thyroid stimulating hormone (TSH) from anterior pituitary gland [8].

2.2 Endocrine dysfunction

Endocrine dysfunction is characterized by abnormal production and secretion of hormones from particular glands. Endocrine dysfunction can be categorized into following types; endocrine hyposecretion (deficiency of hormones), endocrine hypersecretion (excess of hormones), altered tissue response (hormone insensitivity irrespective of circulating hormone) and endocrine tumors [3, 9].

3. Thyroid gland

The thyroid gland is, morphologically, a butterfly-shaped organ, located anterior to the trachea, just inferior to the larynx. It is flanked by wing-shaped left and right lobes and the medial region called isthmus [3, 10]. The thyroid gland produces thyroid hormones, mainly triiodothyronine (T_3) and thyroxine (T_4). Multiple thyroid hormone receptor isoforms, derived from two distinct genes, mediate the action of thyroid hormones. The thyroid hormone receptors belong to a nuclear receptor superfamily. Thyroid hormone receptors bind to specific thyroid hormone-responsive sequences in promoters of target genes by regulating transcription. However, hypothalamic-pituitary-thyroid axis regulates thyroid hormones [7, 11].

3.1 Hypothalamic-pituitary-thyroid (HPT) axis

The hypothalamic-pituitary-thyroid axis is the part of neuroendocrine system consisting of hypothalamus, pituitary gland and thyroid gland. The hypothalamus is directly connected to the pituitary gland [12]. Hypothalamus secretes TRH which stimulates pituitary gland to produce and secrete TSH. TSH then acts on thyroid gland to produce and secrete thyroxine (T_4) and triiodothyronine (T_3). T_4 is converted into T_3 by deidonination controlled by various hormones like TSH, vasopressin and catecholamines in the peripheral organs (liver, adipose tissues, glia

and skeletal muscles). T_4 and T_3 control the secretion of TRH and TSH by negative feedback mechanism to maintain normal levels of the hormones of HPT axis into the blood stream. Reduced levels of circulating TH result in increased TRH and TSH production and vice versa [13].

3.2 Thyroid dysfunction

Thyroid disease is very common worldwide affecting 5–15% of general population. Women are 3–4 times more susceptible to experience any type of thyroid disease. Thyroid dysfunction can be due to overproduction or under production of thyroid hormones. Thyroid disorders can lead to enlargement of thyroid gland as well as thyroid cancer. Abnormal production of thyroid hormones can lead to following pathological conditions; hypothyroidism (under production of thyroid hormones) and hyperthyroidism (overproduction of thyroid hormones) [3, 14]. There are a few drugs, classically associated with thyroid dysfunction, including lithium, amiodarone, interferon alfa, interleukin-2 and tyrosine kinase inhibitors [15].

3.2.1 Hypothyroidism

Hypothyroidism is described as the thyroid gland's inability to produce enough thyroid hormone to meet the body's metabolic demands. Hypertension, dyslipidemia, cognitive impairment, infertility and neuromuscular dysfunction are associated with untreated hypothyroidism. Hypothyroidism is more prevalent in women than men and increases with age. Primary thyroid gland failure or insufficient gland stimulation by the hypothalamus or pituitary gland may lead to hypothyroidism. Primary gland failure can be resulted from congenital abnormalities, iodine deficiency, autoimmune destruction (Hashimoto disease) and infiltrative diseases. Iatrogenic hypothyroidism occurs after radioiodine therapy, thyroid surgery and neck irradiation. Disorders generally associated with transient hypothyroidism include postpartum thyroiditis, silent thyroiditis, subacute thyroiditis and thyroiditis associated with thyroid stimulating hormone (TSH) and receptor-blocking antibodies. Basic causes of hypothyroidism are generally found with other manifestations of hypothalamic or pituitary dysfunction, and, are characterized by decreased levels of TSH relative to inadequate thyroid hormone.

3.2.2 Hyperthyroidism

Hyperthyroidism is defined as "the excessive production and secretion of thyroid hormones from the thyroid gland" and is characterized by weight loss, tachycardia, palpitation, arrhythmia, tremor, nervousness, irritability, anxiety, heat intolerance, sweating, increased thirst and appetite, fatigue, hyperdefecation, diffused goiter, warm and moist skin and disturbances in menstrual cycle [14, 16]. Hyperthyroidism can be caused by graves' disease, painless thyroiditis or postpartum thyroiditis, painful subacute thyroiditis, toxic multinodular goiter or toxic adenoma and exogenous thyroid hormone excess [3]. Menstrual disturbances are common in hyperthyroidism. Thyrotoxicosis may cause delay in sexual maturation and onset of menstrual cycle, oligomenorrhea, polymenorrhea and increased concentrations of sex hormone binding globulin (SHBG). Progesterone (P4) and follicle-stimulating hormone (FSH) significantly increase and, luteinizing hormone (LH) as well as estradiol (E2) significantly decrease in hyperthyroidism [17].

4. Ovary

Ovaries are the female pelvic reproductive organs that house the ova and are also responsible for the production of sex hormones. Ovaries are paired organs located on both sides of the uterus within the broad ligament beneath the uterine (fallopian) tubes. The ovary within the ovarian fossa is a space that is bound by the external iliac vessels, obliterated umbilical artery and the ureter. The ovaries house and release ova or eggs, needed for reproduction. A female has approximately 1–2 million eggs at the time of birth but only 300 of these eggs will become mature and released for fertilization [18].

4.1 Polycystic ovary syndrome (PCOS)

PCOS is the common endocrine disorder among females. It is estimated that 6–10% of women are affected by PCOS in reproductive years of their life. 1 out of 10 women experiences its symptoms in her fertile age. The multifaceted nature of PCOS makes it difficult to define. This clinically heterogenous endocrine syndrome is infertility to gynecologist, hirsutism to a dermatologist, menstrual irregularity to a physician and pseudo-Cushing's disease to an internist. Considering all the the symptoms collectively, it can be defined by hyperandrogenism, oligomenorrhea and multiple cystic follicles in ovaries. Disturbed pulsatile release of GnRH leads to excessive LH, contributing to hyperandrogenism and polycystic morphology. Genetic and epigenetic reasons of these changes have also been investigated [19, 20].

4.2 Hypothalamic-pituitary-ovarian (HPO) axis

Reproductive activity is regulated by the hypothalamic-pituitary-ovarian (HPO) axis which secretes hormones necessary for reproduction. HPO is comprised of three main components. Hypothalamus is located at the base of the brain, just above the brainstem. Along with homeostasis, the hypothalamus also secretes certain hormones, including gonadotropin-releasing hormone (GnRH). Pituitary gland is located below the hypothalamus, in the base of the skull. This gland secretes a variety of hormones, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in response to GnRH. Ovaries are located in the woman's pelvis, and secrete estrogen and progesterone [21].

5. PCOS and hypothalamic-pitutary-thyroid axis

HPO axis and HPT axis are physiologically related. Thyroid receptors in ovaries control female reproductive functions and estrogen affects HPT axis. This link designates subclinical hypothyroidism as a determinant of PCOS. The high prevalence of hypothyroidism among PCOS patients also indicates a strong relation. Thyroid levels are more frequently disturbed in PCOS patients and are more commonly associated with anovulation. Insulin resistance is also a common feature of both the diseases. Incidence of subclinical hypothyroidism among PCOS women augments insulin resistance and hyperandrogenism [22, 23].

6. Prevalence

The autoimmune thyroid disease (AITD) is found more prevalent in females with PCOS than the females without PCOS. Many systematic prospective studies

were carried out to observe the levels of thyroglobulin (Tg) antibodies and thyroid peroxidase (TPO), distinctive for hashimoto thyroiditis (HT) in females with PCOS. It was observed that TPO and Tg levels were elevated in PCOS patients than the healthy females. Moreover, in thyroid ultrasound, hypoechoic pattern which is typical of Hashimoto thyroiditis (HT) was also found more prevalent in PCOS patients. Increased level of thyroid antibodies and hypoechoic thyroid ultrasound pattern revealed the prevalence of HT in PCOS patients and found to be increased by three-fold when compared with controls [24, 25]. In Asia, recently cross-sectional studies, revealed higher prevalence of TPO-positive autoimmune thyroiditis with increased mean TSH levels, increased prevalence of goiter and frequently a hypoechoic thyroid ultrasound pattern in patients with PCOS aged between 13 and 45 years, than in control [1, 26, 27]. Recent meta-analysis included most of the studies, which confirmed higher prevalence of AITD, higher TSH levels and positive TPO and TG antibodies in PCOS patients than in controls [28].

The possibility of having Graves' disease along with PCOS could be higher. In this regard, no broad epidemiological data was found as of recently with the exception of the case reports [1, 2, 29].

In girls of age 13–18 years with HT, a study showed highly significant prevalence of PCOS than in girls without HT, who were negative for TPO antibodies [30]. From the majority of studies, this can be concluded that HT and PCOS frequently occur together.

7. Etiology and pathogenesis

The etiology of HT is complicated and involves mainly genetic along with gender-associated and environmental factors like iodine supply, drugs, chemicals and infections [31]. Similarly, genetic, ovarian-related as well as other hormonal and metabolic factors such as hyperinsulinemia were supposed to involve in the etiology of PCOS [32].

Genetic susceptibility for HT has been confirmed by family and twin studies [33, 34]. Similarly, genetic susceptibility and familial aggregation were also found in PCOS patients [35, 36]. Various susceptibility genes have already been proposed for HT as well as PCOS [37, 38]. Although, a common genetic background still has not been established. Polymorphism of susceptibility genes in HT may influence the occurrence and characteristics of PCOS. Such possible connections will be discussed in more detail. Furthermore, HT is the most prevalent autoimmune disorder [37]. Possible role of autoimmune phenomena in the etiology of PCOS has been suggested [30, 39]. Therefore, supposed genetic and causal factors related to autoimmunity in both the disorders will be explained along with the role of polymorphism of susceptibility genes, alter growth factor beta (TGFβ), regulatory T cells (Tregs), the thymus and variations of sex hormones.

7.1 Susceptibility and candidate genes

In HT, family and twin studies recognized strong genetic susceptibility. The risk of developing HT is increased by 32 and 21 fold in children and siblings of patients with HT respectively, where females were more prone to be affected than males [33]. Various genes are said to be associated with the disease occurrence, progression and severity such as human leukocyte antigen (HLA-DR), cytotoxic

T-lymphocyte-associated protein 4 (CTLA4), CD40, interleukin 2 receptor, protein tyrosine phosphatase 22 (PTPN22), alpha (IL2RA), vitamin D receptor (VDR) and thyroid-specific gene thyroglobulin (Tg) [31, 40, 41].

Familial clustering is well established in PCOS. An increased prevalence of PCOS has been documented in first-degree relatives of females with PCOS [38, 42, 43]. Several candidate genes have been studied for PCOS, such as those coding for fibrillin 3 (FBN3), insulin (INS), INS receptor substrate 1, transcription factor 7-like 2, calpain 10, the fat mass and obesity associated protein [44, 45], sex hormone binding globulin (SHBG) [38] and VDR [46]. Recently, in an Asian as well as European population, the DENND1A gene, which encodes a protein participating in the endosomal membrane transport, was recognized by genome-wide association studies (GWAS) as a true PCOS susceptibility gene [47, 48]. However, the found results of a large number of candidate gene studies were mostly inconclusive.

7.2 Genetic polymorphism

FBN3 gene polymorphisms may play a role in the etiology of PCOS and HT by influencing the activity of TGF, which is regulated by FBNs. The FBN3 gene, like FBN1 and FBN2, is likely to encode FBNs, which are microfibril networks in the extracellular matrix that provide binding opportunities for TGF sequestration [49, 50]. Polymorphisms in the FBN3 gene, which impact the activity of TGF, which is regulated by FBNs, may play a role in the etiology of PCOS and HT. FBN3 is likely to encode FBNs, which are a component of extracellular matrix microfibril networks that provide binding opportunities for TGF sequestration, similar to FBN1 and FBN2 [47, 50–52]. Activins, inhibins, and anti-Mullerian hormone, all members of the TGF superfamily, are thought to play a role in the etiology of PCOS. However, genome wide association studies (GWAS) have found no members of the TGF signaling pathway to be among the top signals for PCOS. Changes in TGF have been linked to the etiology of PCOS in terms of prenatal origins, metabolic abnormalities, and reproductive abnormalities [50]. FBN3 is abundant in fetal organs, including the ovaries [53, 54]. FBN3 expression in the stromal compartments of fetal ovaries disappears after the first trimester. As a result, FBN3 has an effect on the activity of TGF, which is involved in the regulation of stromal formation and function throughout fetal development, confirming notions about PCOS having a fetal origin [54]. Recent genetic studies have also reported that polymorphism of the FBN3 gene has been shown to be associated with the levels of TGFβ. Allele 8 (A8) of D19S884, a dinucleotide repeat polymorphism in intron 55 of the fibrillin-3 gene, is linked to polycystic ovary syndrome [55]. Similarly, in HT, lower levels of serum TGFβ1 were found when compared with healthy controls. Moreover, levels of serum TGFβ1 did not increase after treatment with levothyroxine (l-T_4), indicating the interrelation between TGFβ1 and HT [56]. TGF stimulates the production of the transcription factor forkhead box P3 (FOXP3) and the creation of Tregs in the establishment of immunological tolerance, and it works as a fundamental regulator of immune tolerance by promoting suppressive Tregs and blocking T cell differentiation [31, 57].

As a result, TGF could play a role in the development of autoimmune diseases like HT. Given this context, it's possible that PCOS women with allele 8 of the D19S884 gene in the FBN3 gene, and hence lower TGF1 levels, are more likely to develop HT than PCOS women without allele 8, but this has yet to be researched.

There has recently been evidence of a link between the three prime untranslated region (3′-UTR) mutation rs1038426 of the gonadotropin-releasing hormone receptor

(GnRHR) and INS production in PCOS, as well as a link between serum TSH, serum INS levels, and INS sensitivity. This could point to a significant role for GnRHR genetic variants in INS secretion and INS resistance in PCOS, as well as a link to thyroid function [58].

Finally, the CYP1B1 gene, which codes for an enzyme that converts E2 to 4-hydroxyestradiol, is linked to PCOS. The CYP1B1 L432V (rs1056836) polymorphism was linked to serum thyroxine (T_4), free T_3 (fT_3), and free T_4 (fT_4) levels [59]. This discovery could point to a third genetic relationship between thyroid function and PCOS.

7.3 Thymus

The importance of the thymus gland in immune system modulation and autoimmune development is well understood. Two processes permit the maintenance of self-tolerance and prevention of autoimmunity; the central immunological tolerance, which is enabled by the thymic deletion of autoreactive T cells during fetal development, and peripheral immune tolerance, in which Tregs play the key role [37, 60]. These cells are attained from the thymus as well as peripheral T cells. Tregs suppress the immune system and prevent an overabundance of immunological responses [61]. As previously established, lower TGF1 levels in the blood have been linked to HT [56].

In animal models, estrogen-induced immunological disruption has been demonstrated to play a role in the development of PCOS. Anovulation and follicular cysts were generated in female mice when estrogen was given before 10 days of age, when the thymus was in the latter stages of development [62]. The effect of estrogen on the thymus was investigated in estrogen-injected female mice with intact thymus, had follicular cysts in their ovaries; however, no cysts were found in mice who were thymectomized before estrogen injections and then reconstituted with adult thymocytes. Ovulation occurred and follicular cysts did not arise when estrogen was unable to exert influence upon the thymus during its development when adult thymic cells were given later. In addition, estrogen-injected animals with an intact thymus had a lower number of thymocytes than controls. The absence of Tregs due to an estrogen-affected thymus was thought to be a needed for the production of estrogen-induced cysts, supporting the autoimmune etiology of PCOS [63]. Similarly, the highest prevalence of infertility was seen in women prenatally exposed to diethylstilbestrol (DES), a strong synthetic estrogen that was given in the United States from 1940 to 1971, when they were exposed to DES from 9 to 12 gestational weeks [64]. This is also the period during which the thymus develops at its most rapidly [65]. A higher frequency of autoimmune disorders has been found in DES-exposed women [66]. Phytoestrogens, which are found in flax seeds and soy bean products, may expose modern pregnant women to higher doses of estrogen. In addition to estrogens, adrenal steroids like corticosterone have been demonstrated to reduce thymic weight and number, resulting in anovulation and the production of ovarian cysts in mice [67].

To summarize, different variables such as excessive estrogen levels or severe stress with increased adrenal hormones may be responsible for changes in the fetal thymus, resulting in changes in immunological tolerance and the occurrence of HT and PCOS in predisposed individuals in adulthood.

7.4 Sex hormones

The sex hormones play an important role as females are significantly more often affected by autoimmune disorders than males. Autoimmune disease autoimmune

affects 5% of the world's population and 78% of those affects women [68]. A doubled chromosome X and a low androgen-to-estrogen ratio were thought to play a role in the etiology of autoimmune disorders even in Klinefelter's syndrome [69]. The onset of autoimmune disorders in women is earlier than in males, and it frequently correlates with elevated levels of the female hormone progesterone [68]. As a result, when comparing pre-pubertal children with chronic autoimmune thyroiditis to pubertal adolescents or adults, the female-to-male ratio was shown to be considerably lower in pre-pubertal children with chronic autoimmune thyroiditis [70]. Similarly, estrogen usage was linked negatively with the presence of TPO antibodies [71]. During the menstrual cycle, higher levels of estrogens during the follicular phase and lower levels of estrogens during menstruation and luteal phase, lead to a shift from Th1 to Th2 mediated immunity, respectively [72]. As a result, throughout the typical menstrual cycle, levels of the Th2 cytokine interleukin 6 (IL6) were adversely linked with progesterone levels in young women. IL6 levels were lowest during the luteal phase and highest during the follicular phase [73]. The activation of FOXP3 and the generation of Tregs was inhibited by IL6 [62]. On the other hand estrogens have been shown to promote Treg development [72].

As a result, it was observed that the number of Tregs decreases during the luteal phase and increases during the late follicular phase [74]. Pregnancy causes several changes in the immune system in order to tolerate the fetus, the most notable of which is a shift from Th1 to Th2 cytokine profile [75, 76]. This is most likely due to Treg expansion generated by estrogen, which suppresses both Th1 and Th2 immune responses, while the latter are less vulnerable to Tregs and thus prevail. After delivery, a decrease in Tregs alters the cytokine profile from Th2 to Th1, causing autoimmunity to exacerbate or worsen [76]. A connection between the number of deliveries and the risk of AITD was found in a few retrospective studies [77, 78].

Sex hormones regulate in vitro and in vivo immune system [79]. Estrogens have been linked to a hyperactivity of T cells and a hypoactivity of B cells in animal studies [80]. The generation of autoantibodies was higher in female mice than in male mice [81]. Estrogens have been shown to decrease T suppressor cell function, enhance B cell activity, boost the release of the Th2 cytokine IL6, and shift the immune response to Th2 and antibody generation [38, 68]. In comparison to men, women have a greater CD4+/CD8+ ratio, higher CD4+ levels, and more antibodies [75]. Androgens suppress most immune system components, increase the activity of T suppressor cells, and increase the Th1 response and CD8+ cell activation [74, 82]. Progesterone inhibits macrophage growth, IL6 generation, and peripheral antibody production [82]. Oscillations in progesterone levels during pregnancy and the ovulatory cycle are thought to be linked to reversible immune system alterations [83].

Women with PCOS have lower progesterone and higher testosterone levels than women without PCOS [2]. Menstrual irregularity in women suffering from PCOS and several anovulatory cycles may have no or very low progesterone, resulting in an elevated estrogen-to-progesterone ratio for long duration. As a result, their vulnerability to autoimmune diseases may increases because of a stimulating effect of estrogens on the immune system [39, 49]. On the other hand, autoimmune disease could be prevented by androgens. However, their impact on the immune system and levels in PCOS are unlikely to be sufficient to avoid autoimmunity. As a result, an imbalance in progesterone, estrogen, and androgens may contribute to the development of HT. Taking this idea into account, as well as the three PCOS phenotypes that have been postulated [84], the increased prevalence of HT would be expected in women with

PCOS and chronic anovulation as well as without hyperandrogenism, followed by classic PCOS with hyperandrogenism and anovulation, while the decreased incidence would be supposed to expect in ovulatory PCOS with hyperandrogenism. However, this hypothesis is yet to be confirmed.

8. Conclusions

Almost unanimously, prevalence studies report on a frequent joint appearance of PCOS and HT in women within the reproductive age. Therefore, the above discussion, may conclude that thyroid disorders and PCOS are undoubtedly associated with each other, with respect to their etiology, pathogenesis and clinical consequences. However, this chapter provides scientific ground to further investigate the connection between thyroid dysfunction and PCOS.

Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

AITD	autoimmune thyroid disease
CTLA	cytotoxic T-lymphocyte-associated protein
E	estradiol
FBN	fibrillin
FSH	follicle stimulating hormone
GnRH	gonadotropin releasing hormone
GWAS	genome wide association studies
HPOA	hypothalamic-pituitary-ovarian axis
HPTA	hypothalamic-pituitary-thyroid axis
HT	Hashimoto's thyroiditis
HLA	human leukocyte antigen
IL	interleukin
IL2RA	interleukin 2 receptor alpha
INS	insulin
LH	luteinizing hormone
PCOS	polycystic ovary syndrome
PTPN	protein tyrosine phosphate non-receptor
P	progesterone
SBGH	sex hormone binding globulin
Tg	thyroglobulin
TGFβ	growth factor beta
Th	T helper cell
TH	thyroid hormone
TPO	thyroid peroxidase
TRN	thyrotropin releasing hormone
Tregs	regulatory T cells
TSH	thyroid stimulating hormone

T_3	triiodothyronine
T_4	thyroxine
UTR	untranslated region
VDR	vitamin D receptor

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Chapter 3

Polycystic Ovary Syndrome: It's Not Just Infertility

Naheed Akhter, Sadia Sana, Naila Iftikhar, Muhammad Adnan Ahsan, Abu Huraira and Zafaar Siddique

Abstract

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine issue described by unpredictable menses, hyperandrogenism, and polycystic ovaries (PCO). The commonness of PCOS changes relying upon which measures are utilized to conclude yet is just about as high as **15–20%** when the European culture for human propagation and embryology/American culture for regenerative medication rules are utilized. Clinical signs incorporated grown-ups incorporate sort 1 diabetes, type 2 diabetes, and gestational diabetes. Insulin opposition influences half **70%** of ladies with PCOS prompting a few comorbidities including metabolic condition, hypertension, dyslipidemia, glucose narrow-mindedness, and diabetes. Studies show that ladies with PCOS are bound to have expanded coronary corridor calcium scores and expanded carotid intima-media thickness. Psychological wellness problems including despondency, uneasiness, bipolar turmoil, and voraciously consuming food issues additionally happen all the more habitually in ladies with PCOS. Weight reduction works on feminine abnormalities, indications of androgen abundance, and barrenness the board of clinical appearances of PCOS incorporates oral contraceptives for feminine inconsistencies and hirsutism. Spironolactone and finasteride are utilized to treat indications of androgen overabundance.

Keywords: infertility, PCOS, subfertility, endometrial malignancy, hirsutism

1. Introduction

In 1935, scientists depicted a few ladies giving oligo/amenorrhea joined with the presence of reciprocal polycystic ovaries (PCO) set up during the medical procedure. Three of these seven ladies likewise gave weight, while five gave indications of hirsutism [1–5]. Just a single lady was both fat and showed hirsutism [6]. These discoveries infer that on the off chance that PCO is determined by morphology in ladies to have oligo/anovulation, not every one of the elements which are accepted to be related to PCOS should be present [5, 7]. Moreover, with the utilization of transvaginal ultrasonography, it has become obvious that ladies with oligo/amenorrhea, weight, and hirsutism do not all have the common PCO morphology. The event of significant heterogeneity in clinical indications and endocrine provisions related to polycystic ovary disorder (PCOS) infers that a few ladies with PCO on ultrasound output might even show none of the different elements of PCOS.

Since there is at present no general meaning of PCOS, distinctive master bunches utilize various measures to analyze the condition. In any case, every one of the gatherings searches for the accompanying three components [8, 9].

1. Feminine abnormalities, such as light periods or skipped periods, that outcome from long haul nonappearance of ovulation (the interaction that sets a full-grown egg-free from the ovary).

2. Undeniable degrees of androgens that do not result from different causes or conditions, or indications of high androgens, such as overabundance body or beard growth.

3. Numerous pimples of a particular size on either of the ovaries as identified by ultrasound.

2. Causes of polycystic ovary syndrome

Doctors do not know what causes PCOS. They admit that a high amount of male hormones prevent the ovaries from producing hormones and eggs normally. Genes, insulin resistance, and inflammation have all been associated with excess androgen production [10].

2.1 Genes

Research shows that polycystic ovary syndrome runs in families.
Almost certainly, numerous qualities—not only one—add to the conditions [11].

2.2 Insulin resistance

More than 70% of ladies with polycystic ovary syndrome have insulin opposition, implying that their body cells cannot utilize insulin appropriately. The chemical produces by the pancreas to help the body use sugar from food sources for energy is insulin [12].

At the point when cells cannot utilize insulin appropriately, the interest of the body in insulin increments. The pancreas produces more insulin to redress. Additional insulin activates the ovaries to create more androgen.

The significant reason for insulin obstruction is weight. Corpulence and insulin opposition can expand your danger for type 2 diabetes [13].

2.3 Inflammation

Ladies with polycystic ovary syndrome frequently have expanded degrees of irritation in their bodies. Being overweight can in like manner add to aggravations. Research has connected overabundance irritation to higher androgen levels [14].

3. PCOS dangers

If you have been determined to have polycystic ovary disorder (PCOS), comprehend the drawn-out well-being hazards related to the sickness, which include the following:

1. Fruitlessness or subfertility

2. Endometrial malignant growth

3. Diabetes

4. Lipid anomalies

5. Cardiovascular dangers

6. Obstructive rest apnea

Not all ladies with PCOS will foster these conditions, however, having PCOS expands your danger. Accordingly, have your well-being checked routinely by a doctor who has experience treating ladies with PCOS. Normal doctor visits ought to be booked through your conceptive years and proceed after menopause, even though you will presently do not have sporadic periods and other PCOS manifestations might diminish after the feminine cycle closes [8, 10].

The globally regarded doctors at the middle for polycystic ovary condition supervise the consideration of thousands of ladies with PCOS consistently. UChicago medication is additionally home to specialists in malignancy, coronary illness, and other medical issues who can analyze and treat these conditions if they create [3].

3.1 Fruitlessness or subfertility

Numerous ladies do not understand that they have PCOS until they see a specialist decide why they cannot get pregnant. Fruitlessness or subfertility (diminished richness) is a typical issue for ladies with PCOS.

This might be because of the lopsidedness of chemicals brought about by an overproduction of the male chemical testosterone. The ovaries may inconsistently deliver ova (eggs). Because of the accessibility of ovulation-inciting medications and advances in helped conceptive advances, numerous ladies with PCOS would now be able to imagine [15].

Even though PCOS might diminish a lady's opportunities to become pregnant, the illness is certifiably not a substitute for anti-conception medication. Numerous ladies with PCOS do become pregnant, without clinical help. Ladies who are physically dynamic and do not wish to imagine ought to think about utilizing a prophylactic [16].

3.2 Endometrial malignancy (endometrial carcinoma)

Ladies with PCOS have all the earmarks of being at expanded danger for creating malignant growth of the endometrium (coating of the uterus) further down the road. From your teenagers through menopause, all ladies experience a month-to-month development of the endometrial covering in the uterus, as the body sets itself up for the capability of a treated egg. On the off chance that you do not become pregnant, the coating regularly is shed through the period [17].

Ladies with PCOS likewise experience the month-to-month development of the endometrial covering. Notwithstanding, the covering is not adequately shed since she has rare or nonexistent feminine periods. In this way, the covering proceeds to assemble and can build the danger of endometrial malignant growth [18].

3.3 Diabetes

Insulin assists the body with using or interaction (glucose). Insulin obstruction or weakened glucose resistance have been connected to PCOS. Moreover, significant degrees of insulin invigorate the creation of testosterone, which bothers PCOS [17, 19].

By age 40, up to 40% of ladies with PCOS have some degree of unusual glucose resilience, as one or other diabetes or weakened glucose resistance. Our doctors at UChicago Medication's Middle for Polycystic Ovary Disorder direct continuous exploration on the job of insulin opposition and insulin activity in ladies with PCOS. A lot of this exploration has been distributed in clinical diaries, such as New Britain Diary of Medication and Diary of Clinical Endocrinology and Digestion [20].

3.4 Lipid irregularities

Hyperandrogenism (expanded testosterone) can prompt a troublesome lipid profile in ladies with PCOS. This implies that a lady with PCOS might have an undeniable degree of fat substances in her circulatory system. In certain ladies, the blood lipid profile might show a lower pace of high-thickness lipoproteins (HDL the "Great" cholesterol) and a higher pace of low-thickness lipoproteins (LDL the "Awful" cholesterol). This irregularity builds the danger of cardiovascular sickness [21].

3.5 Cardiovascular dangers

Proof proposes that ladies with PCOS are at expanded danger for coronary illness and other cardiovascular sicknesses. Moreover, the inclination for ladies with PCOS to be overweight expands the danger of cardiovascular sickness, similarly as heftiness increments cardiovascular danger among ladies and men who do not have PCOS [20].

3.6 Obstructive rest apnea

Studies led at the College of Chicago have affirmed the outstandingly high danger of obstructive rest apnea among ladies with PCOS. While expanded body weight adds to this danger, ladies with PCOS appear to be at high danger as an outcome of different elements notwithstanding weight. For instance, the high testosterone levels in PCOS additionally appear to assume a part in the improvement of rest apnea [22].

4. Sign and symptoms

Polycystic ovary condition (PCOS) is a hormonal issue normal among ladies of conceptive age. PCOS indications might start soon after pubescence, yet can likewise create during the later high scholar years and early adulthood. Since indications might be ascribed to different causes or go unrecognized, PCOS might go undiscovered for quite a while. Generally, an analysis of PCOS can be made when you experience two of these **three signs** [23].

4.1 Unpredictable periods

Individuals with PCOS regularly have sporadic or missed periods because of not ovulating. Rare periods are a typical indication of PCOS. For instance, you may have

less than nine periods every year with over 35 days between periods. Different ladies experience the ill effects of strangely weighty periods [24].

4.2 Polycystic ovaries

Albeit certain individuals might foster blisters on their ovaries, many individuals do not. Your ovaries may be developed and contain follicles that encompass the eggs. Accordingly, the ovaries may neglect to work routinely [18, 25].

4.3 Overabundance androgen

Raised degrees of male chemicals might bring about actual signs, such as overabundance of facial and body hair (hirsutism), and sometimes extreme skin inflammation and male-design hairlessness [25, 26].

5. Symptoms

- Default period, sporadic period, or extremely light period
- Ovaries that are prodigious or have many sores
- Overabundance body hair, including the chest, stomach, and back (hirsutism)
- Increase in weight, particularly around the tummy (mid-region)
- Acne or sleek epidermis
- Male-design hairlessness and diminishing hairs
- Infertility
- Little pieces of abundance skin on neck and armpits (skin labels)
- Dull or toughness small areas on the rear of the neck and armpits [27].

6. Indications of polycystic ovary syndrome

6.1 Weight gain

About a portion of individuals with polycystic ovary syndrome will have weight gain and stoutness that is hard to oversee [28].

6.2 Exhaustion

Many individuals with polycystic ovary syndrome report expanded exhaustion and low energy. Related issues, for example, helpless rest might add to the sensation of weariness [29].

6.3 Undesirable hair development (hirsutism)

Regions influenced by overabundance hair development might incorporate the face, arms, back, chest, thumbs, toes, and mid-region. Hirsutism identified with PCOS is because of hormonal changes in androgens [30].

6.4 Diminishing hair on the head

Going bald identified with polycystic ovary syndrome might increment in middle age.

6.5 Fruitlessness

PCOS is the main source of female fruitlessness. Notwithstanding, only one out of every odd lady with PCOS is something very similar. Albeit certain individuals might require the help of fruitfulness medicines, others can imagine normally [31].

6.6 Skin inflammation

Hormonal changes identified with androgens can prompt skin inflammation issues. Male chemicals can make the skin oilier than expected and cause breakouts in regions, such as the face, chest, and upper back [31].

6.7 Obscuring of skin

You might see thick, dull, smooth patches of skin under your arms or bosoms, or on the rear of your neck [32].

6.8 State of mind change

Having polycystic ovary syndrome can improve the chances of emotional episodes, sorrow, and uneasiness [11, 33].

6.9 Pelvic agony

Pelvic agony might happen with periods, alongside weighty dying. It might likewise happen when a lady is not dying [11, 32].

7. How PCOS affects fertility

Polycystic ovarian disorder is the main source of ovulatory fruitlessness. Up to 80% of females who have polycystic ovary syndrome experience related fruitfulness challenges. In case you are experiencing issues getting pregnant, you have an assortment of treatment choices. A certain way of life alterations is the best option to further develop fruitfulness, trailed by prescriptions, hormonal medicines, and helped regenerative strategies [34].

A trademark indication of polycystic ovary syndrome is sporadic or missing feminine periods. Certain individuals with PCOS may not get a period for months, even a

long time, while others will encounter draining for quite some time. A little level of those with polycystic ovary syndrome will encounter month-to-month cycles [35].

Sporadic or missing feminine cycles in polycystic ovary syndrome are because of a fundamental hormonal awkwardness [36].

- Normally, sex chemicals, such as luteinizing hormones (LH), are emitted at a consistent heartbeat rate. In polycystic ovary syndrome, LH is emitted at a fast heartbeat rate.

- The LH emission design conveys messages to the ovaries to siphon out more elevated levels of male chemicals, like testosterone.

- Overabundance LH and testosterone trigger negative input circles, which adjust the arrival of chemicals/hormones that control ovulation and the feminine cycle.

- The follicle that would regularly be delivered to be prepared in pregnancy never completely develops and in some cases, does not get set free from the ovary.

Minuscule follicles show up as a string of pearl on an ultrasound, sometimes encompassing the ovary. These follicles are called cysts/pimples because of their appearance, despite the fact that they vary from the ovarian sores that can develop and crack. Unsuccessful labors are likewise normal with polycystic ovary syndrome and might be because of the imbalance of sex chemicals and more elevated levels of insulin [35, 37].

8. Diagnoses

Medical care suppliers search for three trademark elements of polycystic ovary disorder (PCOS)—nonattendance of ovulation, significant degrees of androgens, and blisters on the ovaries. Having at least one of these components could prompt a finding of PCOS. If your clinical history proposes that you may have PCOS, your medical care supplier will preclude different conditions that might cause comparable manifestations [37, 38].

8.1 Before making a finding of PCOS

8.1.1 Take a full family history

Your medical care supplier will get some information about your feminine cycle and any set of experiences of fruitlessness. The person likewise will find out if you have a mother or sister with PCOS or with manifestations like yours, as PCOS will in general spat families [39].

8.1.2 Conduct a complete physical exam

Your medical care supplier will do an actual test and search for additional hair development, skin break out, and different indications of undeniable levels of the chemical androgen. The individual additionally will take your pulse, measure your

abdomen, and work out your weight list, a proportion of your muscle versus fat dependent on your stature and weight [40].

8.1.3 Take blood tests

Your medical care supplier will look at the degrees of androgens, cholesterol, and sugar in your blood [41].

8.1.4 Do a pelvic test or ultrasound to look at your ovaries

During the pelvic test, your medical care supplier will embed two fingers into your vagina and push on your midsection to feel for blisters on your ovaries. To assist with seeing growths in your ovaries, the individual in question may suggest an ultrasound, a test that utilizes sound waves to snap a photo of your pelvic region. Your medical care supplier likewise will check how thick the coating of your uterus is; if your periods are unpredictable, the covering of your uterus could be thicker than typical.

9. How is polycystic ovary syndrome treated?

Treatment for polycystic ovary syndrome depends on several aspects. These might incorporate your age, how serious your indications are, and your general well-being. The kind of treatment may likewise rely upon whether you need to become pregnant later on [42, 43].

- If you do plan to become pregnant, your treatment may include:

A change in diet and activity

A sound eating routine and more active work can assist you with getting in shape and lessen your indications. They can likewise help your body to use insulin all the more productively, lower blood glucose levels, and may assist you with ovulating [44].

Medications to cause ovulation

Medicine can assist the ovaries with delivering eggs normally. These drugs additionally have specific dangers. They can expand the chances of multiple births (twins or more). What's more, they can cause ovarian hyperstimulation. This is the point at which the ovaries discharge and excessive hormones. It can cause manifestations, for example, stomach bulging and pelvic agony [45].

- If you do not plan to become pregnant, your treatment may include:

Birth control pills

These assist to control periods, lowering androgen levels, and diminishing skin inflammation [44].

Diabetes medication

This is frequently used to bring down insulin opposition in PCOS. It might likewise assist with diminishing androgen levels, slow hair development, and assist you with ovulating all the more routinely [46, 47].

A change in diet and activity

A healthy diet and more exercise can help you to reduce weight and your indication. They can also assist your body to utilize insulin more efficiently, diminish blood glucose levels, and may help in ovulating [48].

Medications to treat other symptoms

A few drugs can assist with lessening hair development or skin inflammation [47].

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Chapter 4

Rare and Underappreciated Causes of Polycystic Ovarian Syndrome

Alan Sacerdote

Abstract

While hyperinsulinemia is a common contributing mechanism in the pathogenesis of polycystic ovarian syndrome (PCOS), other mechanisms may give rise to or add to the effects of hyperinsulinemia, as well as other causes of hyperandrogenism, in the pathogenesis of PCOS. Such underappreciated causes may include autoimmune, insulin receptor mutations, mutations of post-receptor insulin signaling response elements, polymorphisms of LH, androgen, and estrogen signaling pathways, epigenetic alterations in hormonal signaling cascade response elements, infestations and infections with organisms capable of endocrine disruption by various mechanisms, as well as drugs and other chemicals which may be endocrine disruptors. In addition, alterations in the gut, oral, or vaginal biome may be associated with PCOS and insulin resistance and may, in some instances, have a role to play in its pathogenesis. In this chapter I plan to review what is known about these lesser-known causes of PCOS, in the hopes of alerting clinicians to consider them and stimulating investigators to better understand PCOS pathogenesis in general and, hopefully, develop more individualized, precision treatment and prevention strategies for the people in our care.

Keywords: PCOS, insulin resistance, biome, polymorphisms, epigenetic, endocrine disruptors, autoimmune, vitamin D

1. Introduction

Polycystic ovarian syndrome (PCOS) is believed to be the most common cause of infertility in reproductive age women [1]. Although men lack ovaries, there is also a syndrome called male PCOS featuring a similar set of cardiometabolic indicators and risks to that seen in female PCOS as well as early balding [2]. Most people with PCOS are insulin resistant/hyperinsulinemic [3]. When overweight or obesity is present in people with PCOS (as is the case in most people with this disorder) insulin resistance/hyperinsulinemia is exacerbated [3]. Several mutations are associated with an increased risk of PCOS [4]. Gene methylation and histone acetylation abnormalities as well as certain non-coding RNAs may also contribute to the expression of PCOS [5].

Autoimmune disease has been shown to play a role in some people with PCOS, severely insulin resistant diabetes, acanthosis nigricans, and systemic lupus

erythematosus with nephritis [6]. Besides these rare and dramatic examples of the Type B syndrome, there is some evidence that an autoimmune process triggered by low progesterone levels may be a trigger for PCOS in many women [7]. A similar phenotype without lupus or anti-insulin receptor antibodies, but with insulin receptor mutations or abnormal post-receptor signaling has also been described [6].

Parasitoses, which are especially common in the tropical and subtropical parts of the world (and becoming more common as a result of the climate crisis), can induce PCOS by virtue of their ability to synthesize steroid hormones, e.g., estradiol and 1,25-OH2-vitamin D3 from its precursor, 25-OH-vitamin D [8].

Alterations in the microbiome have been reported in people with PCOS which may play a causative role [9–11].

Endocrine disruptor chemicals (EDCs), which are ubiquitous and increasing in our environment, including certain drugs, may also contribute to the pathogenesis of PCOS [12, 13].

Epilepsy has been cited as a cause of PCOS, however, there is controversy as to whether the disorder itself, treatment with valproate, or both are responsible [14].

Many drugs are associated with an increase in insulin resistance (IR) which may be an initial step in PCOS pathogenesis. In addition, EDCs in our environment may initially cause IR or bind as agonists to estrogen or androgen receptors, eventually contributing to PCOS [15].

In the remainder of this chapter, I shall review what is known about these diverse contributing causes of PCOS in the hope that in so doing clinicians might explore these often-reversible factors in their patients. I further hope that such a review may point to common pathogenic pathways in many, if not all, people with PCOS. Finally, I hope that appreciation of the various causes of PCOS can lead to improved preventive strategies and individualized, precision treatment of people with PCOS.

2. Genetic predisposition to PCOS

The marked tendency of PCOS for familial clustering (made even more remarkable by the hypo-fertility of people with PCOS) has long supported the notion that PCOS has a genetic component. However, since families often share similar diets, lifestyles, and EDC exposures, twin studies using monozygotic twins raised in very different environments would be helpful in separating genetic and environmental effects. Although it was shown that the tetrachoric correlation for PCOS in monozygotic twin sisters is higher than for dizygotic twins or for non-twin sisters, each set of twins or sisters in this large study was brought up in the same family. Despite efforts of most parents to raise monozygotic twins as distinct individuals, they are apt to, nonetheless, share a more similar environment and set of experiences than less closely related siblings, leaving open the possibility that shared environment/experience contributes significantly to the correlation [16]. In addition to tetrachoric correlation, both univariate analysis and a trivariate genetic analysis of major findings occurring in women with PCOS suggested a strong genetic component of PCOS in this Dutch twin study by Vink and colleagues [16]. Other twin studies in people with PCOS have reached similar conclusions [17–20].

Genome-wide association studies (GWAS) have been helpful in identifying polymorphisms that are associated with an increased risk of PCOS development [21–24]. Nevertheless, only about 10% of the apparent heritability of PCOS to date can be explained by these associations, leading to speculation that various phenotypes

are associated with rare polymorphisms. Newer technologies e.g., gene and whole exome sequencing may clarify the contribution of rare polymorphisms to different phenotypes in the future [21].

Among the GWAS-identified candidate loci are DENND1A, LHCGR, INSR, FSHR, ZNF217, YAP1, INSR, RAB5B, and C9orf3 [22]. Polymorphisms found in DENND1A (P = .0002), THADA (P = .035), FSHR (P = .007), and INSR (P = .046) in Chinese women with PCOS were also strongly associated with PCOS in European women [24].

GWAS often fails to identify candidate loci in the mitochondrial portion of the genome [25]. Recent publications suggest that the mitochondria may play a pivotal role in PCOS pathogenesis, both genetically and epigenetically, given the essential mitochondrial role in cellular metabolism and IR. Recently Ye and colleagues reported that a 4977 base pair deletion in mitochondrial DNA detected in peripheral blood using multiplex probe-based qPCR was highly associated with PCOS in a logistic regression analysis [26]. In a study by Saeed and colleagues it was reported that most of the mitochondrial DNA mutations (80%) were limited to a 3157–3275 base region which is evolutionarily conserved and would be expected to change the secondary structure of mitochondrial transfer RNAs. As suspected, 6 mutations (A to G and/or T to C) altered the expected base pairing. Mitochondrial DNA copy numbers were also diminished in women with PCOS compared with controls [27]. Zeng et al. have reviewed the role of oxidative stress (OS) in people with PCOS [28]. They summarized much of what is currently known about the role of mitochondrial dysfunction in PCOS. Reduction of mitochondrial DNA copy number and mitochondrial mutations contribute to IR, metabolic syndrome, and disordered development of ovarian follicles through increased production of reactive oxygen species (ROS). Obesity plays a pivotal role in the pathogenesis of PCOS in most people, however, mitochondrial genome alterations related to PCOS with obesity are not yet well understood, underlining the need to investigate changes in the mitochondrial genome that are associated with obesity. External environmental factors may also disrupt mitochondrial function. Recent attention has focused on the effect of environmental factors e.g., cigarette smoke and bisphenol A on reproduction. Cigarette smoke has been reported to disrupt ovarian development; 1-(N-methyl-N-nitrosamino)-1-(3-pyridinyl)-4-butanal (NNA), contained in third-hand smoke, reduced ovarian weight and follicle number in rats exposed to NNA for 30 days compared with controls and even had a serious negative effect on development of the offspring of NNA-exposed rats. These adverse reproductive effects of cigarette smoke seem to be due to mitochondrial dysfunction. NNA exposure causes ROS buildup by increasing superoxide dismutase (SOD) mRNA levels, inducing apoptosis. Benzo(a)pyrene (BaP), another component of cigarette smoke, causes massive mitochondrial ROS leakage/dysfunction, resulting in significant plasma membrane lipid peroxidation and disrupted ovum fertilization. Cigarette smoke also adversely effects the development of granulosa cells, which have an essential role in providing optimal amounts of the hormones and nutrients needed for follicular development.

3. Epigenetic contributions to PCOS

These include abnormalities of DNA methylation, histone acetylation, and downstream signal transduction abnormalities.

3.1 Abnormalities of DNA methylation and histone acetylation

Epigenome-wide association studies (EWASs) are helping in the discovery of environmentally mediated molecular changes in PCOS from disease pathogenesis to the discovery of epigenetic markers. Recent epigenetic studies offer persuasive evidence linking epigenetic regulation with PCOS etiology, presentation, clinical phenotypes, and comorbidities, which could potentially lead to improved disease prevention and management via precisely targeted strategies. Several pivotal biological pathways have been repeatedly reported by independent groups, supporting functional regulation by endocrine abnormalities and metabolic dysfunction in PCOS, while also suggesting an autoimmune component in the syndrome [29]. Increasing application of high-throughput sequencing technologies for epigenome analysis combined with evidence-based causal inference should facilitate precision PCOS prevention/treatment in the future.

Vázquez-Martínez et al. recently reviewed the topic of DNA methylation in women with PCOS [5]. Alterations in DNA methylation, histone acetylation and non-coding RNAs have been found in diverse tissues of women with PCOS. DNA methylation abnormalities appear in peripheral and umbilical cord blood, and in ovarian and fat tissue of women with PCOS, suggesting a pivotal role for these epigenetic modifications in the pathogenesis of this disorder. Possibly, these derangements in DNA methylation facilitate deregulation of gene expression involving inflammation, hormone biosynthesis and signaling, as well as glucose and lipid metabolism. The authors have compiled an extensive table of the tissues in which methylation abnormalities are encountered in women with PCOS indicating whether the involved DNA is hypo- or hypermethylated, the changes in gene expression, if any, related to the methylation variants, and any documented clinical/phenotypic expression resulting from these changes. Interestingly, both hypomethylation of some genes and hypermethylation of others may predispose to PCOS.

3.2 Epigenetic effects of hyperandrogenism

Qu and colleagues studied the effects of hyperandrogenism on the expression of histone deacetylase 3 (HDAC3), peroxisome proliferator-activated receptor gamma 1 (PPARG1), and nuclear corepressor 1 (NCOR1) genes in the granulosa cells of women with a hyperandrogenic form of PCOS, compared with women with non-hyperandrogenic PCOS, women without PCOS who had tubal infertility, and a rodent model of PCOS [30]. NCOR1 and HDAC3 mRNA expression was higher in the hyperandrogenic women than in normo-androgenemic women with PCOS and controls ($P < 0.05$). When all women were divided into successful and failed pregnancy subgroups, they found lower PPARG1 mRNA levels and higher NCOR1 and HDAC3 mRNA levels in the failed subgroup with hyperandrogenic PCOS ($P < 0.05$). Two hypermethylated CpG loci in the PPARG1 promoter and 5 hypomethylated CpG loci in the NCOR1 promoter were encountered only in the hyperandrogenic women with PCOS ($P < 0.01$–$P < 0.0005$). The acetylation levels of histone H3 at lysine 9 and p21 mRNA expression were low in human granulosa cells cultured with dihydrotestosterone in vitro ($P < 0.05$). A PCOS rodent model also displayed abnormal PPARG1, NCOR1, and HDAC3 mRNA expression and methylation alterations of PPARG1 and NCOR1, consistent with those found in women with hyperandrogenic PCOS. A strength of this study is the consistent effect of hyperandrogenism in the induction of epigenetic changes in PPARG1, NCOR1, and HDAC3 in granulosa cells in hyperandrogenic

women and rodents with PCOS as well as in vitro, which have a role in the ovarian dysfunction encountered in women with a hyperandrogenic PCOS phenotype.

4. Parasitosis as a cause of PCOS

When considering our genome and our epigenome we often lose sight of the fact that the organisms that live within us and on us, though having a different number of chromosomes than the cells we think of as human with somewhat different gene sequences, contribute to our total genome and epigenome. In sheer number, the cells of our biome far exceed the number of cells we think of as human. The character and density of their gene products profoundly influence our hormonal, metabolic, and immune milieu, and even our mood and personality. In the case of parasites, they are in turn hosts to biomes of their own.

As mentioned in the Introduction, we have published the case history of a woman who had PCOS associated with extensive neurocysticercosis [8]. She had refused standard treatment with albendazole for her parasitosis (which she presumably acquired in her native Mexico) because of fear of drug side effects that some of her affected friends had experienced. She had been referred to our clinic because of complaints of worsening hirsutism and amenorrhea x 2 years. She was 32 years old G1P1001. Diagnostic work-up fulfilled Rotterdam criteria for PCOS with amenorrhea, hirsutism, low sex hormone binding globulin, and an elevated LH/FSH ratio. Non-classic adrenal hyperplasia, pregnancy, and virilizing tumors were excluded by appropriate tests. Hypovitaminosis D was excluded by measurement of vitamin D metabolites, however, her serum 1,25(OH)2-vitamin D3 level was elevated. Treatment with lifestyle modification (weight loss diet, prescribed exercise), and gradually uptitrated doses of metformin to 2000 mg/day was associated with a gradual reduction in hirsutism and a return of menses, although still with oligomenorrhea. SHBG rose slightly and there was normalization of the LH/FSH ratio.

We wanted to know whether her extensive burden of neurocysticercosis was playing a role in the etiopathogenesis of her PCOS, perhaps by pressing on the GnRH cells of the hypothalamus, however, the neuroradiologist could find no evidence of anatomic hypothalamic involvement by the encysted parasites. We also considered the possibility that her elevated serum 1,25-(OH)2-vitamin D3 elevation was due to the formation of granuloma-like lesions around the encysted parasites with either the encysted parasites or the surrounding mononuclear cells synthesizing 1,25(OH)2-vitamin D3 in excess, as occurs in other granulomatous disorders like pulmonary sarcoidosis and tuberculosis. We also performed a literature search for associations between cysticercosis and PCOS. While we did not find any reports of such an association, we did learn that Taenia sp. prefer female to male hosts, and pregnant to non-gravid hosts [31–33]. It was later learned that Taenia sp. have steroidogenic enzymes and can synthesize steroid hormones e.g., estradiol [34–39]. As the cysticercosis burden increases, the host, whether female or male, will be further estrogenized, rendering the host milieu more favorable to the parasites. While PCOS is correctly considered a hyperandrogenic condition in most women, it is also important to remember that it is also a state of unopposed estrogen effect in anovulatory or oligo-ovulatory women. The sustained estrogen effect would be conducive to Taenia parasitization and increasing cysticercosis burden. In addition, Taenia sp. can metabolize the relatively weak androgen, androstenedione, to the more potent androgen, testosterone [34].

In searching further, we learned that the selective estrogen receptor modulator (SERM), tamoxifen, had successfully reduced cysticercosis burden in a murine model [40]. Since our patient continued to decline standard treatment for cysticercosis we offered her a trial of treatment with another SERM, raloxifene, which did not carry the risk of estrogenic endometrial stimulation reported with tamoxifen [41]. We thoroughly reviewed the article by Vargas-Villavicencio et al. with our patient and carefully explained that raloxifene was an approved and generally safe drug in the US for the treatment of post-menopausal osteoporosis/osteopenia, but not for neurocysticercosis. We explained that it was similar to, but distinct from and safer than the tamoxifen used in that article. We emphasized the importance of avoiding conception during the trial using abstinence or reliable barrier contraception as this was an FDA Category X drug (should not be used in pregnancy). We obtained her informed consent and initiated treatment with raloxifene at the standard dose for osteoporosis/osteopenia of 60 mg/day. When she returned to clinic, about 7 weeks after starting raloxifene, she related that she thought she might be pregnant and that, despite being forewarned, she had had unprotected intercourse on a few occasions. Pregnancy was confirmed by physical examination and serum HCG level, and she was counseled on her options. She elected to terminate her pregnancy. Following termination, a repeat brain MRI was performed. It was read by the same neuroradiologist who had read her baseline study. He was blinded regarding her treatment between the 2 studies. On the repeat study the total number of encysted lesions fell from 37 to 33, 10 lesions shrunk, 5 disappeared, 18 were unchanged, 4 enlarged and 1 new lesion appeared. Subsequently, after the patient belatedly agreed to and underwent standard treatment with albendazole and dexamethasone, serum 1,25-(OH)2-vitamin D3 fell from 81 to 41 pg/ml while 25-OH-vitamin D level only fell from 34 to 30 ng/ml. This reduction in calcitriol level occurred even though dexamethasone has been reported to increase the serum concentration of this metabolite [42].

This was the first case to be reported of human neurocysticercosis wherein modification of the hormonal milieu was associated with a reduction of cestode burden. The pregnancy on raloxifene, though unfortunate, supported the concept that neurocysticercosis contributed to the pathogenesis of her PCOS. Serum 1,25-(OH)2-vitamin D3 may ultimately prove to be a useful biomarker for assessing disease activity in neurocysticercosis, as it is in several other granulomatous disorders [43]. This report and the preclinical reports preceding it conceptually opened the field of biome contribution to endocrine disorders.

5. The Biome in the pathogenesis and maintenance of PCOS

Yurtdaş and Akdevelioğlu recently reviewed the literature on the gut biome and PCOS [44]. While genetic, neuroendocrine, epigenetic and metabolic factors are reported to contribute to the pathogenesis of PCOS, knowledge of the etiologies of the syndrome(s) remains incomplete. Recently, studies in humans and preclinical models have found associations between alterations in the gut microbiome and the metabolic/clinical features of PCOS.

It is theorized that gut dysbiosis could be a pathogenetic factor in PCOS. Accordingly, changing the gut microbiome using probiotics, prebiotics, and synbiotics as well as diet may serve as a new therapeutic modality for PCOS. Specific changes of the gut microbiome in women with PCOS are apparently associated with distinct PCOS phenotypes. Several recent studies indicate that IR, sex steroid concentrations,

and obesity alter the quantity, diversity and species composition of gut bacteria in women with PCOS (and vice versa).

Liang and colleagues studied gut biome dysbiosis in PCOS in association with obesity [45]. They recruited 8 obese women with PCOS, 9 lean women with PCOS, and 9 lean control women. Gut bacterial composition was assessed by PCR. Obese women with PCOS were found to have lower observed bacterial structural variants (SVs) and alpha diversity (a composite of different measurements that estimate diversity in a single sample) than the control group, higher beta diversity (a measure of the similarity/dissimilarity of 2 communities) than the lean PCOS group ($P < 0.05$), and lower abundances of genera (particularly butyrate producers). Regression analysis demonstrated that decreased abundances of several bacterial genera correlated with higher serum testosterone and impaired glucose tolerance. PCOS was associated with alterations in the gut microbiome population. Obesity appears to have a critical role in the development of a dysbiotic gut microbiome in women with PCOS.

Lindheim et al. studied associations between changes in the gut microbiome composition and gut barrier function and metabolic and reproductive abnormalities in women with PCOS [46]. Gut microbiome composition was assessed in stool samples from women with PCOS (n = 24) and healthy control women (n = 19) using 16S rRNA gene amplicon sequencing. Processing of data and microbiome analysis were performed in mothur and QIIME utilizing differing relative abundance cut-off points. Integrity of gut barrier function, inflammation, and endotoxemia were assessed using serum and stool indicators. Correlations with anthropometric, metabolic, and reproductive measures were then calculated. The stool microbiome of women with PCOS demonstrated lower bacterial species diversity and an altered phylogenetic mix compared with controls. The authors did not find significant differences in any bacterial taxa with a relative abundance>1%. Among rare bacterial taxa the relative abundance of those from the order ML615J-28 (phylum Tenericutes) and from the family S24-7 (phylum Bacteroidetes) was significantly lower and was associated with unfavorable reproductive parameters in women with PCOS. Women with PCOS showed alterations in some, but not all markers of gut barrier function and endotoxemia.

Women with PCOS had less species diversity and an altered phylogenetic mix in their stool microbiome, which was associated with certain adverse clinical parameters. Gut barrier malfunction and endotoxemia were not pivotal factors in these women, however, they may contribute to the particular phenotype seen in some people with PCOS.

Given the accumulating data that the gut biome population contributes to the etiopathogenesis of PCOS it seems intuitive that "normalizing" the gut biome in the most rapid way possible, fecal transplant from a "healthy" woman to a woman with PCOS, might effect the most rapid amelioration of the syndrome with the least risk. Such an approach has been dramatically successful in treating pseudomembranous colitis [47]. Although there are no human studies to date, a small study assessing fecal transplant to treat PCOS in a rodent model has been reported with encouraging results [48]. This same study also found amelioration of PCOS in the model with isolated Lactobacillus transplantation.

While a relatively short term improvement in the gut biome is usually sufficient to treat antibiotic dysbiosis-related conditions like pseudomembranous colitis, more chronic conditions, like PCOS, metabolic syndrome, Type 2 diabetes, and inflammatory bowel disease seem to require long term lifestyle changes e.g. shifting from a Western-style diet high in sucrose, animal fat, and animal protein to a prebiotic/probiotic rich,

lower calorie, phytonutrient-rich, mostly plant-based diet and an increased amount of regular exercise in order to sustain the improved gut biome and remission of the disorder being treated [49, 50]. Plant-based diets of this type are accompanied by reduced inflammation, less gut permeability, reduced generation of reactive oxygen species (ROS), and improved insulin sensitivity.

5.1 Drugs which alter the gut biome

Certain drugs, chiefly those used to treat obesity, prediabetes, and T2DM are known to alter the gut biome favorably, while others, the best known of which are antibiotics, may cause dysbiosis with unfavorable metabolic consequences [51–53]. Among the drugs with beneficial gut biome effects which explain at least part of their clinical actions are metformin, the alpha-glucosidase inhibitors, the GLP-1 receptor agonists, and the dual GLP-1/GLP-2 receptor agonist, tirzepatide.

5.2 Bariatric surgery and the gut biome

In addition to diet and drugs, bariatric (metabolic) surgery may affect the gut biome [54]. The taxonomic make-up of the gut bacterial microbiome is significantly affected by metabolic surgery. The most frequent alteration reported in most preclinical and human studies is a relative decline in abundance of Firmicutes with an increase in Bacteroidetes, Proteobacteria, and its class Gammaproteobacteria (order Enterobacteriales, family Enterobacteriaceae, genus Escherichia). Interestingly, the gut microbiome population differs substantially in rodents and humans. Proteobacteria increase after metabolic surgery due to a higher gut lumen pH and higher levels of dissolved oxygen that favor growth of facultative aerobic bacteria and inhibit growth of anaerobic bacteria. Reduction in stomach volume after bariatric surgery increases luminal gastric and distal gut pH, resulting in altered bacterial populations and overgrowth. More alkaline gut pH favors growth of *Akkermansia muciniphila*, *Escherichia coli*, and Bacteroides spp. which are species more typical of the oral microbiome. The greater bacterial diversity postoperatively includes increases in the phyla Verrucomicrobia and Fusobacteria, and a lower proportion of Actinobacteria. It is interesting that the use of metformin is also associated with an increased growth of Akkermansia [55].

5.3 Alterations in the vaginal biome are also associated with PCOS

While far more research has been reported on the contributions of the gut biome to the pathogenesis and maintenance of PCOS, recently the possible role of the vaginal biome in PCOS has come under scrutiny [10]. Hong and associates obtained vaginal swabs from 39 women with recently diagnosed PCOS and 40 women without PCOS and compared them using 16S rRNA gene sequencing in a case control study. Screening values for possible bacterial biomarkers of PCOS were analyzed by receiver operating characteristic (ROC) curve methodology. There were significant differences in the vaginal biome bacterial populations between the 2 groups. The vaginal bacterial species in the PCOS group were more diversified than those in the control group (Simpson index of the PCOS group vs. the control group: median 0.49 vs. 0.80, $P = .008$; Shannon index: median 1.07 vs. 0.44, $P = .003$; Chao1 index: median 85.12 vs. 66.13, $P < .001$). This is in marked contrast to what has been reported for the gut biome, which is less diverse in women with PCOS, obesity, and T2DM than in healthy

control women. Relative abundance of *Lactobacillus crispatus* in the stool samples of the women with PCOS was significantly lower than in healthy controls (P = .001), and relative abundance of Mycoplasma and Prevotella was higher than in healthy control women (P < .001, P = .002, respectively). Adjustments for BMI and vaginal cleanliness grade did not change these associations. Genus Mycoplasma may be a biomarker for PCOS screening, since ROC analysis showed that the area under the curve (AUC) for relative abundance of Mycoplasma was 0.958 (95% CI, 0.901–0.999).

5.4 The oral cavity biome and PCOS

The oral cavity biome has also been explored in terms of PCOS [11]. This study was designed to investigate the hypothesis that the concentrations of suspected periodontal pathogens in saliva and the host serum antibody response is elevated in women with PCOS, compared with healthy controls. In total, 125 women in 4 groups were studied: 45 with PCOS+healthy periodontium, 35 with PCOS+gingivitis, 25 systemically and periodontally healthy women, and 20 systemically healthy women with gingivitis.

Salivary concentrations of 7 suspected periodontal pathogens were analyzed by quantitative real-time PCR, while serum antibody titres were measured by ELISA. In women who had PCOS, salivary populations of *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Streptococcus oralis* and *Tannerella forsythia* levels were higher than in matched systemically healthy women, especially when gingivitis was also present. PCOS was also associated with increased *P. gingivalis*, *Prevotella intermedia*, and *S. oralis* serum antibody titres if gingivitis was present. The most consistent effect appeared to be the increased population of and antibody response to *P. gingivalis*.

In my search I could not find any reports of associations of the skin, aural, or nasal/sinus biomes with PCOS.

Although newer technologies e.g., 16S rRNA are a giant step forward in our understanding of biome/systemic disorder interactions, it is important to understand that the study of biomes is still in its infancy. Our microbiomes include viruses, fungi, prions, protozoa, and sometimes parasites, and algae. Future research will doubtless uncover important associations between these organisms/pre-organisms and systemic disorders like PCOS.

6. Endocrine disrupting chemicals and PCOS

Endocrine disrupting chemicals (EDCs), both environmental and drug, appear to contribute to the etiopathogenesis of PCOS. This may occur via binding to sex hormone receptors or by causing IR/hyperinsulinemia; additional mechanisms are also possible.

Environmental EDCs-In our species increased serum bisphenol A (BPA) concentrations have been reported in teenagers and women with PCOS compared with reproductively healthy controls and these are positively correlated with androgen levels, suggesting a role for this chemical in the etiopathogenesis of PCOS, although causality is yet be established [56–60]. It is possible that embryonic/fetal exposure to certain EDCs permanently changes reproductive, neuro-endocrine, and metabolic regulation favoring PCOS development, in genetically predisposed people, or hastening and/or exacerbating the natural course of the disorder via lifelong exposure.

In pre-clinical studies, exposure of mothers to BPA changes postnatal development and sexual maturation in the offspring. Exposure to dibutyl phthalate and

di(2-ethylhexyl)phthalate during pregnancy results in polycystic ovaries and a hormonal profile similar to that seen in human PCOS. Androgenic EDCs, nicotine, and 3,4,4′-trichlorocarbanilide, all contribute to the creation of a concerning hyperandrogenic embryonic/fetal milieu. Prenatal EDC exposure may contribute to abnormal embryonic/fetal developmental programming and partially explain the wide variability in PCOS phenotype.

Research has mostly focused on the possible roles of the most widely distributed and studied environmental agents suspected of contributing to the etiopathogenesis of PCOS. Plasticizers, including BPA and phthalates, which are known EDCs, and advanced glycation end products (AGEs) are ubiquitous in our milieu; therefore, our attention should be focused on reducing such exposure. The timing of EDC exposure is critical for understanding the diversity and severity of adverse health consequences. Embryos/fetuses, infants, and young children are the most vulnerable groups. Prenatal EDC exposure that imitates some actions of endogenous hormones may contribute to abnormal fetal programming and, ultimately, result in PCOS and other adverse health consequences, possibly even trans-generationally. Acute or more protracted EDC exposure and dietary (mostly from Western type diets), as well as endogenously formed AGE exposure in different stages of the life cycle can alter the hormonal milieu and result in disruption of reproductive function. AGEs are proinflammatory molecules capable of interacting with cell membrane receptors and mediate triggering of proinflammatory signaling pathways and oxidative stress. These agents may also contribute to metabolic changes, e.g., obesity, IR, and the compensatory hyperinsulinemia that can create or worsen the PCOS phenotype and contribute to its complications, e.g., Type 2 diabetes and cardiovascular disease. Prediabetes and T2DM both result in hyperglycemia, leading to the formation of even more AGEs in a vicious cycle [61, 62].

Large population surveys find countless chemicals in our serum and tissues that did not even exist in our grandparents' generation [60] Sadly, regulatory agencies are losing the race to evaluate these compounds for safety before they are released into our environment.

7. Drugs which may contribute to the pathogenesis of PCOS

In addition to the EDCs which accidentally find their way into our bodies, many prescription drugs may also contribute to the etiopathogenesis and maintenance of PCOS [61]. Most of the drugs which contribute to causing PCOS do so by causing IR/hyperinsulinemia. In so doing they often contribute to causing other disorders associated with IR, including metabolic syndrome, T2DM, hypertension, gout, dyslipidemia, and congenital adrenal hyperplasia [62, 63]. Among these drugs are some of the beta-blockers, thiazides and related diuretics, like indapamide, some of the inhibitors of the renin-angiotensin system, nicotinic acid, the fluoroquinolones (which may also contribute by causing bacterial dysbiosis), protease inhibitors, nucleoside reverse transcriptase inhibitors, antipsychotic drugs, especially atypical antipsychotic drugs, divalproex, and high estrogen oral contraceptives.

8. Role of vitamin D in PCOS

The role of vitamin D and polymorphisms in its receptor have been the subject of considerable research, given that vitamin D deficiency has been associated with

IR [63–68]. Vitamin D has a physiologic role in female reproduction, which includes ovarian follicle development and luteinization, by regulating anti-Mullerian hormone (AMH) signaling, follicle-stimulating hormone (FSH) sensitivity, and progesterone biosynthesis in granulosa cells. Vitamin D also affects glucose homeostasis via diverse routes. The evidence for an important role for vitamin D on glucose metabolism includes: the presence of vitamin D receptors in pancreatic β-cells and skeletal muscle, the expression of 1-α-hydroxylase enzyme in these tissues which catalyzes the 1-α-hydroxylation of 25-hydroxy vitamin D (25(OH)D) to 1,25-dihydroxyvitamin D, as well as the presence of a vitamin D response element in the human insulin gene promoter region. About 67–85% of women with PCOS have vitamin D deficiency. While there is no significant difference in serum 25(OH) D concentrations between women with PCOS and controls, a high prevalence of vitamin D deficiency is reported to be associated with metabolic syndrome.

Hypovitaminosis D may worsen the signs and symptoms of PCOS, such as IR, ovulatory and menstrual perturbations, infertility, androgen excess, obesity and increased risk of cardiovascular disease. Many observational reports support a role for vitamin D in an inverse association between women's vitamin D status and metabolic disturbances in PCOS, however, it is difficult to reach a conclusion regarding causality because of contradictory findings from various individual studies and from a recent meta-analysis.

Supplementation of vitamin D reduces abnormally elevated serum AMH concentrations and raises serum anti-inflammatory soluble receptor for AGEs in women with both vitamin D-deficiency women and PCOS. Notably, vitamin D and calcium added to metformin in women with PCOS and vitamin D deficiency improves menstrual regularity and ovulatory rate.

Low serum 25(OH)D concentrations are significantly associated with IR in women with PCOS, leading to suggestions that genes regulating vitamin D metabolism could be candidate genes for PCOS susceptibility. Certain polymorphisms in the vitamin D receptor (VDR) gene including: Cdx2, Taq1, Bsm1, Apa1, and Fok1, have been reported to play an important regulatory role on insulin secretion and sensitivity in women with PCOS. The VDR Fok1 polymorphism was found to have a protective effect against the risk of Type 2 diabetes mellitus, while the Bsm1 polymorphism augmented the risk of Type 2 diabetes. The Apa1 polymorphism has been reported to reduce the risk of vitamin D deficiency [65].

A study was carried out in India, to investigate the association pattern of 4 VDR polymorphisms (Cdx2, Fok1, Apa1 and Taq1) with PCOS among Indian women. They reported a significant difference in genotype and allele frequency distributions of the Cdx2 polymorphism between women with PCOS and controls. A significantly higher frequency of the heterozygous GA genotype and the A allele of Cdx2 was encountered in control women when compared to those with PCOS ($P < 0.001$), suggesting that this single nucleotide polymorphism (SNP) affords some protection against PCOS development. Following adjustment for the covariates of BMI and age, the carriers of the GA genotype and the A allele remained relatively protected against PCOS development. No other significant associations were encountered between the remaining 3 VDR polymorphisms (Fok1, Apa1 and Taq1) and PCOS. They also investigated associations between VDR genotypes and some PCOS clinical/biochemical characteristics and reported that the Cdx2 genotypes were significantly associated with serum testosterone levels while the Fok1 polymorphism showed a significant association with infertility. In addition, the 2 haplotypes made up of 4 polymorphisms, ACCA and ACTA, were also significantly associated with PCOS risk [64].

In a group of Austrian women with PCOS, the VDR Cdx2 polymorphism was found to be associated with higher insulin sensitivity, and the Apa1 polymorphism was associated with lower serum testosterone concentrations. Nevertheless, other investigators did not report any significant differences in the VDR gene polymorphism frequencies between women with PCOS and controls [65].

In a study from Taiwan, it was found that the VDR 1a promoter polymorphisms were not associated with the risk of PCOS but were associated with serum 25(OH)D levels. This study also found that significantly lower serum 25(OH)D levels were seen in women who carried the heterozygous 1521CG/1012GA haplotype of the VDR 1a promoter polymorphisms in both women with PCOS and controls. However, metformin was only able to increase serum 25(OH)D concentrations in women with PCOS who carried the homozygous 1521G/1012A haplotype [65].

Even though several polymorphisms in the VDR gene have been implicated in the etiopathogenesis and presenting phenotype of PCOS, there is considerable heterogeneity in reports from both individual investigators and meta-analyses. Therefore, the role of these VDR gene polymorphisms in the pathogenesis of IR and PCOS remains controversial [65].

Future research with large, independent cohorts and with diverse ethnic populations may clarify whether the associations between vitamin D and PCOS are ethnicity-specific or have differing thresholds depending upon the influence of other individual genotypes in women with PCOS.

A recent reanalysis of data from the D2d trial by the original study authors, using a Cox proportional hazards model, concluded that daily vitamin D intake, sufficient to achieve and maintain a serum 25-(OHD) level $\geq$ 100 nmol/l, is a promising approach to reduce the risk of T2DM in adults with prediabetes, in contrast with their original conclusion, that vitamin D administration was not effective in the prevention of T2DM in those with prediabetes [67, 68].

9. Autoimmunity contributing to the etiopathogenesis of PCOS

In addition to the Type B syndrome of severe insulin resistance, acanthosis, SLE with nephritis, & PCOS discussed in the Introduction, several other autoimmune disorders are associated with PCOS [69, 70]. These include vitiligo, alopecia areata, and the autoimmune polyglandular syndrome. Autoimmune thyroid disease, especially autoimmune (Hashimoto's) thyroiditis, is about 3x more common in women with PCOS compared with controls [70]. Among the reasons cited for these associations are the sustained high estrogen/progesterone ratios in women with PCOS, which prenatally derail embryonic/fetal thymic development and disrupt thymic function as regards preservation of immune self-tolerance, vitamin D deficiency/insufficiency and VDR gene polymorphisms, as well as similarities in the gut biome in people with PCOS and autoimmune disorders, such as increase in those species causing more gut permeability and a reduction of overall bacterial species diversity. These biomic changes are also seen in obesity, metabolic syndrome, and T2DM. In addition, 3 genetic polymorphisms have been reported as predisposing to both PCOS and Hashimoto's thyroiditis. They are polymorphisms of the genes for gonadotropin releasing hormone receptor, fibrillin 3, regulating the activity of transforming growth factor-β and regulatory T cell levels, and CYP1B1 affecting estradiol hydroxylation.

10. PCOS resulting from insulinoma or nesidioblastosis

Murray and colleagues reported PCOS in association with an insulinoma, which resolved following successful removal of the tumor [71]. My literature search did not find any reports of nesidioblastosis-associated PCOS, however, it is predictable, given their chronic hyperinsulinemia, that such individuals will eventually be found.

11. Insulin resistance is not global in PCOS

While there is evident insulin resistance in people with PCOS in terms of carbohydrate, lipid, and uric acid metabolism, there is also evidence of normal or even increased insulin action in features such as hyperandrogenism, acanthosis nigricans, acrochordons, organomegaly, and visceral obesity.

There are 2 major signaling pathways through which insulin's actions are expressed: one signaling cascade is used to regulate intermediary metabolism while the other modulates growth and cell division as well as the hypothalamic/pituitary, gonadal and adrenocortical axes. Regulation of these 2 distinct cascades may be dissociated and data suggest that the activity of the signaling pathway which governs intermediary metabolism is decreased in people with PCOS, T2DM, metabolic syndrome, gout, and congenital adrenal hyperplasia, while the pathways modulating growth processes and mitoses is normal or even enhanced [72]. Most of the intermediary metabolism pathway is activated by insulin binding to its own receptor followed by phosphorylation of IRS-1 and IRS-2. Some of the pathway regulating growth and cell division is initiated by insulin binding to IGF-1 receptors. Even though insulin has greater affinity for its own receptor, when insulin levels are high its receptor is downregulated, limiting available binding sites, so that "excess" insulin will bind to the IGF-1 receptor as an agonist, mimicking the effects of growth hormone. When activation of the IGF-1 cascade is extreme it is sometimes referred to as pseudo acromegaly [73]. Studies show that insulin's signaling pathways normally regulate cell growth, metabolism and survival via activation of mitogen-activated protein kinases (MAPKs) and phosphotidylinositide-3-kinase (PI3K). Activation of PI-3K-associated with insulin receptor substrates-1 and -2 (IRS1, 2) and the subsequent Akt → Foxo1 phosphorylation cascade plays a pivotal role in regulating nutrient homeostasis and organ survival. Several mechanisms have been suggested as causes contributing to the development of IR and metabolic syndrome. These include genetic polymorphisms of proteins in the insulin signaling cascade, suboptimal fetal nutrition, and increased intra-abdominal fat. IR develops as the key player in a cluster of cardiovascular/metabolic dysfunctions we now recognize as metabolic syndrome, which may result in T2DM, a distinctive (Type IV, Fredrickson) dyslipidemia with high VLDL, low HDL, and normal-moderately elevated LDL, accelerated atherosclerosis, hypertension, or congenital adrenal hyperplasia depending on the genetic/epigenetic background of the person with IR including the genetic/epigenetic characteristics of our relevant biomes, vitamin D status, and the influence of drugs and environmental chemicals with endocrine disruptor effects. Inactivation of Akt and activation of Foxo1, via suppression of IRS1 and IRS2 in different tissues following hyperinsulinemia, metabolic inflammation, and overnutrition could be the mechanisms leading to metabolic syndrome in our species [74].

IR in women with PCOS seems to be associated with exaggerated serine residue phosphorylation of insulin receptor substrates. An enzyme extrinsic to the insulin receptors, quite possibly a serine/threonine kinase, causes this aberration and exemplifies a key mechanism for induction of human IR related to extrinsic factors regulating insulin receptor signaling. Serine phosphorylation seems to regulate the activity of P450c16, the pivotal regulatory enzyme in androgen biosynthesis. It is very possible that a single defect results in both IR and hyperandrogenism in some women with PCOS. This IR is selective, affecting glucose/lipid metabolism, but not cell division or growth [75].

12. Sleep disorders and PCOS

It has been reported that women with PCOS have significantly higher risk of obstructive sleep apnea (OSA). OSA severity is significantly correlated with plasma glucose and insulin levels and homeostasis model assessment for insulin resistance (HOMA-IR)-index in women with PCOS. It appears that the progressive worsening of PCOS results in OSA which, in turn, exacerbates the metabolic disturbances, such as IR, associated with this syndrome [76].

Clinic-based studies report that sleep disturbance and disorders such as OSA and excessive daytime sleepiness are more frequently encountered among women with PCOS. Data from the few published population-based studies is substantially concordant. Women with PCOS are mostly overweight/obese, however, this fact only partially explains their sleep problems as significant associations persist after adjusting for body mass index; sleep issues also occur in lean women with PCOS. There are several, likely bidirectional, pathways through which PCOS and sleep disturbances are associated. PCOS pathophysiology includes hyperandrogenemia, a unique form of IR, and possible changes in cortisol and melatonin secretion, plausibly reflecting hypothalamic-pituitary-adrenal dysfunction. Psychological/behavioral factors probably also play a role, such as anxiety and depression, tobacco use, alcohol use, and insufficient exercise which are also frequent among women with PCOS, likely in response to their symptoms. The effects of sleep disturbances on the health of women with PCOS is not completely understood, however, both PCOS and disordered sleep are associated with worsening long term cardiometabolic health and augmented T2DM risk. Immediate quality of life and long-term health status of women with PCOS will likely improve from timely diagnosis and comprehensive management of sleep disorders [77].

13. Light pollution as a contributing cause of PCOS

Several investigators have reported that exposure of rats to continuous light can induce PCOS; however, hyperandrogenism, a key feature of human PCOS, has not been reported previously. In Kang et al.'s article they reported that (a) body weight declined in female rats in continuous light conditions with both ovarian and uterine augmentation; (b) the estrous cycle in rats living in continuous light was disordered, and PCOS-like changes were noted accompanied by hair loss and lethargy; and (c) serum testosterone levels rose significantly in rats living in continuous light. Their results suggest that continuous light can lead to PCOS in female rats without the need for drugs. Poor sleep habits, faulty sleep hygiene, and light pollution may be important contributors to the pathogenesis of PCOS [78].

Dominoni and colleagues as well as others have described reproductive hardships in free-living wildlife associated with light pollution [79].

14. Noise pollution and reproduction

In addition, human-generated noise pollution has been implicated in reduced reproductive success in wildlife, although the mechanisms involved are not clear [80].

15. Undiagnosed non-classic adrenal hyperplasia (NCAH)

Based on my years in clinical practice and academia, I hope readers will indulge me in a personal gripe. When applying the Rotterdam criteria for the diagnosis of PCOS many clinicians ignore or only pay lip service to the exclusions which must be considered an essential part of these criteria. These include thyroid disease, Cushing's syndrome, androgen-secreting neoplasia, hyperprolactinemia, and non-classical congenital adrenal hyperplasia. In my referral practice I found, in reviewing the referral or the written or electronic medical records of patients referred to me for PCOS treatment, that these conditions, especially NCAH had very seldom been excluded by the referring colleague. In the PCOS research literature many investigators do not mention exclusion of these disorders in their PCOS cohorts. In many other articles a single morning unstimulated serum 17-OH-progesterone is proffered as excluding NCAH. The best articles offer a cosyntropin-stimulated 17-OH-progesterone to exclude this diagnosis. In my readings I have not yet encountered a study where NCAH was thoroughly excluded with genetic testing for 21-hydroxylase deficiency as well as cosyntropin stimulation of 17-OH-progesterone, 17-OH-pregnenolone, 11-deoxycortisol, deoxycorticosterone, corticosterone, and 18-OH-corticosterone. Thus, without fully testing for NCAH, most of us have the impression that PCOS is very common and NCAH, except in high-risk ethnic groups is very rare. This is concerning because NCAH and PCOS are often phenotypically identical. However, since therapies aimed at decreasing IR, normalizing the menstrual cycle, reducing androgen secretion or expression, and inducing ovulation are often able to ameliorate both conditions the real-world consequences of misdiagnosis of PCOS may not be as grave as we might expect [63]. Carbunaru and colleagues have reported that the common, non-classic or phenotypic form of 3-beta-ol dehydrogenase deficiency (3-beta-HSD) controlling the adrenal/ovarian synthesis of this enzyme is not associated with an exonic polymorphism, but is associated with IR, hyperandrogenism, and a PCOS phenotype, which in severe forms is called Hyperandrogenism, Insulin Resistance-Acanthosis Nigricans (HAIR-AN) syndrome [81]. It is possible, that a polymorphism may exist in the promoter region of the gene, as has been reported in a group of Brazilian women with non-classic 21-hydroxylase deficiency [82]. Alternatively, several epigenetic modifications could be downregulating the expression of the gene.

16. Lipodystrophies as a cause of PCOS

Lipodystrophies are associated with PCOS due to insulin resistance, which is intrinsic to the lipodystrophies [83, 84].

17. Conclusions

In this chapter I have tried to highlight truly rare contributing causes of PCOS, like insulinomas, as well as showcasing causes that are not particularly rare, but are very rarely considered in clinical practice. The latter include: biomic alterations, epigenetic disturbances such as disordered DNA methylation and/or histone acetylation, and EDCs, including many drugs which contribute to IR. In addition, I have described some very rarely reported causes, like cysticercosis, which, given its extensive global endemicity, will likely turn out to be much more common causes of PCOS than is currently recognized. In exploring this topic, I hope that I have shed some light on common pathways by which these diverse agents might contribute to the etiopathogenesis and maintenance of PCOS, mostly by causing IR/hyperinsulinemia, hyperandrogenism, chronic inflammation, or unopposed estrogenic effects. It is hoped that clinicians will consider these causes more often when evaluating their patients and considering treatments. In so doing, it is likely that better treatment results can be achieved. It is already possible for individual clinicians and their patients to achieve much with interventions such as lower calorie, plant-based diets, supplementation with pre- and probiotics, exercise, ensuring adequate vitamin D status, and choosing drugs with favorable effects on the biome. In addition, patients once educated, may be able to improve their therapeutic outcomes by minimizing their exposure to EDCs in plastics, self-care products, and household products. Major improvements in outcomes may result from efforts at the community, regional, national, and international levels to improve diets, increase exercise, and reduce our exposure to EDCs, light and noise pollution. Attention to sleep hygiene by patients and providers may further reduce the burden of PCOS, metabolic syndrome, resistant hypertension, and T2DM. Fecal transplantation may jump start amelioration of PCOS, provided it is followed with sustained lifestyle changes including plant-based diets, exercise, and possibly pre- and probiotic supplementation. Looking toward the future, the experience we have gained in developing mRNA vaccines against COVID-19 might be applied to develop mRNA "vaccines" against gene products whose overabundance is contributing to PCOS. A fragment of the mRNA could be used to synthesize a fragment of the peptide different enough from the native protein to provoke an adaptive immune response.

Our understanding of the biome and of epigenetics is still in its infancy. As more is learned the opportunities for precision prevention and treatment will increase.

Conflict of interest

The author declares no conflict of interest.

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Chapter 5

Special Considerations on Hyperandrogenism and Insulin Resistance in Nonobese Polycystic Ovaries Syndrome

Tatyana Tatarchuk, Tetiana Tutchenko and Olga Burka

Abstract

PCOS is a widespread phenotypically inhomogeneous endocrinopathy with significant health consequences and incompletely elucidated pathogenesis. Though visceral adiposity and insulin resistance (IR) is a well-proved pathogenic set of factors of PCOS, not all women with obesity and IR have PCOS and not all PCOS women are obese and have IR, which is explained by certain genetic backgrounds. The reported prevalence of nonobese PCOS (NonObPCOS) is about 20–30%, but it may be higher because especially in lean women with nonclassical phenotypes PCOS diagnosis is often delayed or unrecognized. Unlike obese PCOS, NonObPCOS management is less clear and is limited to symptomatic treatment. This chapter presents in structured fashion the existing results on the prevalence of NonObPCOS, as well as on special aspects of body composition, IR, and hyperandrogenism pathogenesis, including adrenal contribution in NonObPCOS.

Keywords: hyperandrogenism, adrenal androgen precursors, insulin resistance, adipokines, hepatokines, steatohepatosis, visceral adiposity, body composition

1. Introduction

Today with the use of Rotterdam diagnostic criteria (at least two of three are present—oligo-anovulation, clinical/biochemical hyperandrogenism (HA), polycystic ovarian morphology (POM) on ultrasound when other causes of these conditions are excluded) polycystic ovary syndrome (PCOS) is the most widespread endocrine disorder in women affecting their reproductive and cardio-metabolic health lifelong [1–5]. PCOS prevalence among reproductive-aged women is from 8 to13% depending on the population ethnicity and diagnostic criteria used [3]. A meta-analysis published in 2017 showed such proportions of PCOS prevalence (95% CI) according to the diagnostic criteria of the National Institute of Health (NIH), Rotterdam criteria, and Androgen Access PCOS Society (AE-PCOS)—6%, 10%, and 10%, respectively. When only unselected population studies were included, the given rates were 6%, 9%, and 10% [6]. Same year meta-analysis of PCOS prevalence in different ethnic groups showed the lowest prevalence in Chinese women (Rotterdam criteria: 5.6%), Caucasians

(NIH: 5.5%), Middle Eastern (NIH 6.1%; Rotterdam 16.0%; AE-PCOS 12.6%), and Black women (NIH: 6.1%) [7]. Despite intensive investigations PCOS etiology remains unclear, relations between its known pathogenic mechanisms are contradictory and consequently the effectiveness of overall management is suboptimal leading to patient dissatisfaction [8]. The reason for this is the high heterogenicity of PCOS in terms of complex genetic background, involvement of developmental origins, and consequently various combinations of pathogenetic mechanisms and clinical features [9, 10].

Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome 2018 confirmed the idea of the 2012 international working group on the need of defining PCOS phenotypic forms based on combinations of diagnostic criteria in research and clinical practice. Thus, there are four phenotypic forms of PCOS—classic (A) involving all three diagnostic criteria, incomplete classic (B), ovulatory (HA and POM only) (C), and normoandrogenic (anovulation and POM only) (D). Back in 2012, much hope was relied on studying PCOS pathogenesis in different phenotypes, but to date, this approach resulted in no definitive answers on details of pathogenesis in different phenotypic forms. It was shown by numerous studies that classic phenotypes are more often associated with obesity, significant visceral adiposity, metabolic syndrome (MS), lifetime risks of type 2 diabetes mellitus (TDM), and cardiovascular disease (CVD) [11–13]. At the same time, ovulatory and normoandrogenic phenotypes seem to be more metabolically safe primarily because of a lower incidence of obesity [12–14]. Though this observation is not universal. Moreover, recent studies showed that while obesity in PCOS universally leads to metabolic complications, hyperandrogenic non-obese women with PCOS (nonObPCOS) also have serious metabolic disturbances, such as nonalchocholic fatty liver disease (NAFLD), dyslipidemia, hyperinsulinemia, and age-related complications like TDM and CVD, in spite of the absence of obvious risk factors [15].

Apart from androgen excess and hypothalamic-pituitary-gonadal axis disfunction, there are two other gross pathogenetic factors of PCOS not included in diagnostic criteria—insulin resistance (IR) with compensatory hyperinsulinemia and ectopic fat distribution with adiposopathy [16, 17]. All these factors are very much interconnected and the question of which of them is primary is still unclear. This can be explained by the fact that the primary factors of encircled pathogenic mechanisms are different in different subgroups of PCOS patients and probably change with time.

The role of overall and especially visceral adiposity is well established in overweight and obese PCOS women [18–21]. Weight reduction is an effective therapeutic approach both for fertility and menstrual function improvement and metabolic risks reduction in overweight/obese women with PCOS [22–25]. But this is not the case with NonObPCOS women. In the scientific aspect, the absence of the main driver of glucose and fat metabolism disruption (obesity) makes NonObPCOS a different pathogenetic subtype of the syndrome.

Reviews summarizing data on NonObPCOS were published in 2017 [26, 27]. In this chapter, we analyze older and recent data on epidemiology, body composition, pathogenesis of insulin resistance, and hyperandrogenism in NonObPCOS.

2. Definition, epidemiology of nonobese PCOS and clinical issues of this population

NonObPCOS gained more researchers' attention after the introduction of Rotterdam criteria. This term emerged spontaneously and there is still no clear-cut

definition of professional societies, guidelines, or consensus statements. Most authors use the criteria of BMI under 25 kg/m^2 and address it as "normal weight PCOS" or "lean PCOS" or "nonobese PCOS" (NonObPCOS) [28]. In some sources, NonObPCOS is addressed as women with PCOS having BMI under 30 kg/m^2 [29]. In this case, it includes both the category of overweight women (BMI 25–29) and normal weight (BMI 18–24). There is data on rare cases of underweight PCOS (BMI < 18), which has to be carefully differentiated with hypothalamic amenorrhea [30, 31]. In this chapter, NonObPCOS will be addressed as any PCOS phenotypic form with BMI < 25 kg/m^2. Lack of a clear-cut definition of NonObPCOS using BMI criteria leads to inconsistent results on its prevalence in different populations. We did not observe studies focused specifically on the prevalence of NonObPCOS. Most available data on the prevalence of nonObPCOS comes from studies on the prevalence of PCOS in different populations or studies targeting metabolic derangements of PCOS and having BMI stratification in their design (**Table 1**). As follows from **Table 1**, the portion of NonObPCOS even with the use of NIH criteria in older studies varies from 20 to 76% [39, 44]. With the use of Rotterdam criteria, the percentage of NonObPCOS varies from 41 to 75% [29, 32–36, 38, 40–43]. Heterogeneity in studies' methodology, participants age, and other factors certainly influence the accuracy of these figures, but still depicts the fact that NonObPCOS is not a minority in this syndrome. Of note is that a greater proportion of NonObPCOS cases is observed in nonselective studies compared to clinical ones when (cohorts of women seeking medical help for hirsutism, menstrual irregularity, etc.).

Data from a meta-analysis by Lizneva et al. [45] supports the notion of the underestimated prevalence of NonObPCOS, probably more often associated with nonclassic phenotypes. The aim of this paper was to evaluate the prevalence of PCOS phenotypes and obesity among patients detected in referral versus unselected populations. The prevalence of more complete phenotypes in PCOS and mean BMI was higher in subjects identified in referral versus unselected populations, suggesting the presence of significant referral bias. The authors analyzed 41 eligible studies. Pooled estimates of detected PCOS phenotype prevalence were consequently documented in referral versus unselected populations, as phenotype A, 50% (95% confidence interval [CI], 46–54) versus 19% (95% CI, 13–27); phenotype B, 13% (95% CI, 11–17) versus 25% (95% CI, 15–37); phenotype C, 14% (95% CI, 12–16) versus 34% (95% CI, 25–46); and phenotype D, 17% (95% CI, 13–22) versus 19% (95% CI, 14–25). Differences between referral and unselected populations were statistically significant for phenotypes A, B, and C. Referral PCOS subjects had a greater mean BMI than local controls, a difference that was not apparent in unselected PCOS [45].

In the setting of the Endocrine gynecology department, Kyiv, Ukraine preliminary patients' database analysis from 2012 to 2021 shows the prevalence of 64% NonObPCOS among all referral PCOS patients (including primary visits of symptomatic patients and referrals from primary care gynecologists because of difficulties in making the diagnosis). We suggest that such prevalence of NonObPCOS in our fourth level institution is caused by uncertainties primary care doctors face in diagnosing PCOS in lean patients especially with mild HA or nonclassical phenotypes as well doubts of patients in the correctness of the diagnosis. With these patients, we often observe interesting phenomena of "not being prone to gaining extra weight" and "having no need to control their calorie intake", which might be a presentation of a "specific type of metabolism worth deeper investigation in terms of metabolic consequences." Thus, available data on NonObPCOS prevalence shows, that this condition is not rare, but likely to be underdiagnosed or diagnosed with delay.

Author, year	Country	Design, PCOS diagnostic criteria	*n*	Age	%BMI <25	%BMI >25
Neubronner (2021) [32]	Singapore	Prospective cross-sectional, healthy women health screen, Rotterdam Criteria	134	21–45	46*	54***
Jena (2021) [33]	India	Hospital-based observational, prospective, Rotterdam Criteria	251	20–40	43.6*	66.4 (BMI ≥25–29-62.94; BMI > 3 0–3.5)
Tosi (2017) [34]	Italy	Retrospective analysis of symptomatic women referred outpatient, Rotterdam Criteria	375	18–45	45.9*	54.1 (BMI ≥25–29-18.9; BMI > 3 0–35.2)
Rashidi (2014) [35]	Iran	Cluster sampling method, NIH, Rotterdam, AE Criteria	602	18–45	41*	59 (BMI ≥25–29-36.9; BMI > 3 0–22.1)
Lauritsen (2014) [36]	Denmark	Prospective, cross-sectional, employees, Rotterdam, AE Criteria	447	20–40	69.9*	31.1 (BMI 25–29 ≥ 16.2; BMI > 3 0–14.9)
Musmar (2013) [37]	Palestine	Cross sectional, students, NIH Criteria	137	18–24	60	30
Li (2013) [38]	China	Epidemiological, 10 provinces, Rotterdam Criteria	159 24	19–45	65.9*	34.1***
Gill (2012) [39]	India	Cross sectional, students, NIH Criteria	152 0	18–25	76*	24***
Yildiz (2012) [29]	Turkey	Cross-sectional, employees, NIH, Rotterdam, AE Criteria	392	18–45	**	**
Moran (2010) [40]	Mexico	Prospective cross-sectional, volunteers, NIH, Rotterdam Criteria	150	20–45	66	34
Chen (2008) [41]	China	Observational with a parallel study, unselected, Rotterdam, AE Criteria	915	20–45	75	25
Azziz (2004) [42]	USA	Prospective, preemployment exam, AE Criteria	400	18–45	32	68 (24 – BMI > 30; 42 BMI > 25)
Asuncion (2000) [43]	Spain	Prospective, blood donors, Rotterdam Criteria	154	18–45	60	40
Michelmore (1999) [44]	England	Cross-sectional observational, volunteers, NIH	230	18–25	20*	80

**Figure obtained by subtraction of the percentage of BMI > 25.*
***Non-obese (<30 kg/m^2) NIH -75.0%; Rotterdam 84.6% AE-PCOS 85.0%. Obese (≥30 kg/m^2) NIH -25.0%; Rotterdam 15.4% AE-PCOS 15.0%.*
****Criterion of ≥27 kg/m^2 was used for obesity and < 23 kg/m^2 for normal weight.*

Table 1.
Prevalence of NonObPCOS.

Today it is obvious that BMI is not an accurate marker of metabolic health since not only adipose tissue excess but more its distribution plays role in metabolic complications, giving the basis for A. De Lorenzo classification—normal weight obese; metabolically obese normal weight; metabolically healthy obese; and metabolically unhealthy obese or "at risk" obese [46, 47]. Thus, while the presence of elevated BMI has a significant positive predictive value for metabolic risks normal BMI does not guarantee their absence since they can be caused by the predominance of ectopic fat distribution and adiposopathy. This fact is considered by most studies of PCOS metabolic aspects discussed below. Studies considering body composition and fat distribution are also inhomogeneous in methodology as will be shown below. In addition, the more accurate methods of body composition evaluation are used the smaller the groups are.

3. Body composition in NonObPCOS, specifics of adipose, and muscle tissue function

In the case of NonObPCOS, we think it is reasonable to analyze body composition data in the first place, as it may have the key to a paradox—of keeping normal BMI despite the presence of predisposing factors, such as HA and IR, and at the same time developing metabolic consequences. Recent studies on bidirectional Mendelian randomization analyses state that increased BMI is causal for PCOS while PCOS is not predictive of obesity [48, 49]. This finding puts even more questions on obese and nonobese PCOS pathogenesis. One of the interpretations can be that high BMI in PCOS is a factor exacerbating epigenetically determined features of the syndrome, such as HA, OD, and IR. This notion is supported by studies demonstrating the presence of IR in most PCOS women irrespective of BMI, though it is positively correlated with BMI. The similar association can be observed for HA—more mild forms of HA are observed in NonObPCOS compared to PCOS with obesity [11, 16, 34]. Taken together these facts shifted research focus from fat mass to the role of the functional state of muscles and different adipose tissue compartments in PCOS pathogenesis. Today adipose tissue (AT) is a recognized player of endocrine, paracrine, and even neurocrine cross-talks, being a target tissue of pancreatic and steroid hormones, source of numerous adipokines, and a place of sex steroids conversion [50]. Visceral AT (VAT) demonstrates more endocrine/paracrine actions [17, 51]. Skeletal muscles are also among the key target organs of pancreatic hormones and sex steroids as well as an important player in metabolism [13]. Thus, studies on body composition's role in and tissue-specific effects of insulin action, androgen synthesis, and lipid turnover seem to be most perspective, especially in the case of NonObPCOS.

Most studies on the body composition of PCOS women were done using anthropometric characteristics that lack accuracy compared to imaging methods (MRI, CT). This led to the formation of the dogma of visceral adiposity in PCOS, which is being debunked by 2019 meta-analysis that using golden standards MRI or CT found no significant difference in accumulations of visceral fat, abdominal subcutaneous fat, total body fat, trunk fat, and android fat in PCOS compared to BMI matched controls. At the same time, meta-regression and subgroup analyses showed that young and non-obese patients were more likely to accumulate android fat [52]. The authors of the paper note the problem of small sample size in studies using gold standard methods for body composition assessment.

Studies on body composition in NonObPCOS in relation to endocrine dysfunctions are limited. In a cross-sectional study of Indian nonobese and obese PCOS women assessed by DXA-scan, higher total body fat, truncal fat, and estimated VAT compared to their age- and BMI-matched controls were reported. Corrected estimated VAT difference was not significant between obese and nonobese PCOS women suggesting that nonobese PCOS women had a similar amount of VAT as that of obese PCOS women when adjusted for their body weight. Also, this study reports that NonObPCOS (overweight and normal weight) were less insulin resistant when compared to the obese PCOS group and postulate that there may be factors other than IR that make the nonobese PCOS women have more VAT, such as postprandial dysglycemia caused by intracrine intestinal factors [53].

Earlier studies of SAT topography in PCOS women using optical devices demonstrated significantly lower total SAT development with a slightly lowered amount of body fat in the upper region and a highly significant leg SAT reduction [54, 55].

In a prospective cohort study of six normal-weight PCOS women and 14 age- and BMI-matched normoandrogenic ovulatory controls, an association of HA with preferential intra-abdominal fat deposition and an increased population of small subcutaneous (SC) abdominal adipocytes was shown. Authors hypothesize that such distribution could constrain SC adipose storage and promote metabolic dysfunction [56]. *In vitro* studies showed that cultured subcutaneous abdominal adipocytes from women with PCOS have diminished insulin-induced glucose transport, reduced insulin receptors content, and decreased insulin-stimulated serine phosphorylation of glycogen synthase kinase (GSK)-3β [57, 58]. Further investigations of SAT-specific features in NonObPCOS by the Dumesic group discovered more details of these compartments' role in PCOS-related dysmetabolism. A prospective cohort study including ten normal-weight women with PCOS and 18 control subjects matched for age and BMI demonstrated that NonObPCOS women have increased adipose-IR and altered adipose stem cell gene expression related to HA and IR [59]. The fact that the number of small adipocytes is stable from early childhood suggests the possibility that SC abdominal adipose expandability through the generation of new small adipocytes is programmed in early life and subsequently becomes insufficient to meet the metabolic demands of most normal-weight women with PCOS. We did not find studies on birthweight, prematurity, and puberty details focusing specifically on NonObPCOS but they might be of great interest. Results of prospective cohort study show accelerated SAT abdominal adipose stem cell differentiation into adipocytes *in vitro* favors sensitivity to insulin *in vivo*, suggesting a role for HA in the evolution of metabolic thrift to enhance fat storage through increased cellular glucose uptake [60].

The role of local androgen conversion in the regulation of abdominal SAT morphology and function is not yet clear in NonObPCOS. Overexpression of aldo-keto reductase 1C3 (AKR1C3)-mediated testosterone (T) generation from androstenedione (A4) promotes local triglyceride (TG) storage in SAT, potentially protecting against lipotoxicity and IR. One study showed that elevated serum T to A4 ratio was a biomarker of subcutaneous abdominal AKR1C3 activity that improved metabolic function in NonObPCOS [61].

Summarizing the existing limited data on AT distribution and function in NonObPCOS, it can be concluded that these women have a predominance of dysfunctional VAT and specific features of SAT limiting its lipid storage capacity. This puts NonObPCOS in the category of normal weight obese or metabolically obese. Metabolic significance of VAT is explained by the following facts—its location in the mesentery and omentum causes drainage directly through the portal circulation to

the liver; the dominance of large or hypertrophic adipocytes and infiltration with immune cells; intensive vascularization and innervation; high density of androgen and glucocorticoid receptors; higher sensitivity to lipolysis and adrenergic stimulation and lower sensitivity to insulin; greater capacity to generate free fatty acids and to uptake glucose, circulating free fatty acids (FFA), and TG [62]. The impaired ability of SAT to store abundant lipids as well as SAT excess leads to accumulation of lipids in atypical sites (liver, skeletal muscles, and even pancreas), known as lipotoxicity phenomena. Lipotoxicity has detrimental effects on a molecular level—endoplasmatic reticulum and mitochondria damage with reactive lipid peroxides (endoplasmatic reticulum stress). The latter can eventually lead to cell apoptosis. At the same time, high levels of circulating FFA leads to a vicious circle of deepening glucose dysmetabolism by limiting blood glucose uptake in AT and muscles [63, 64]. Another effect of AT dysfunctional state in PCOS is altered synthesis of adipokines. Upregulated levels of mRNA levels of the proinflammatory cytokine tumor necrosis factor (TNF) in PCOS reflecting a state of chronic low-grade inflammation in SAT that could lead to low adiponectin were reported [65]. Later independent of BMI and IR decrease in high molecular weight adiponectin in PCOS was demonstrated [66].

The etiology of the described specifics of body composition and AT dysfunction most likely takes roots in genetics and epigenetics. In 2016, Kokosar et al. reported a number of genes and pathways that are affected in adipose tissue from women with PCOS as well as some specific DNA methylation pathways that may affect mRNA expression [67].

Though skeletal muscles also belong to insulin-sensitive organs and normally can utilize up to 70–80% of blood glucose, there are far less studies on specific features of their function in PCOS. There are a lot of debatable aspects to this topic. While osteosarcopenia was reported in obese PCOS no such studies are available for NonObPCOS [68]. On one hand, studies from sports medicine report a positive effect of higher physiological androgen levels on muscle performance as well as superior performance of mildly hyperandrogenic women in sports [69]. On the other hand, peripheral IR documented by euglycemic hyperinsulinemic clamp test in the majority of obese and NonObPCOS suggests the presence of some insulin signaling defect similar to that of TDM [70, 71]. Recent studies by N. Stepto and Hansen suggest that this defect is located in the distal part of the insulin signaling pathway but there may also be additional mechanisms [71, 72]. Hansen suggests that reduced expression and activation of AMP-activated protein kinase (key regulator of glucose uptake in muscle) is due to low levels of adiponectin [72]. Infiltration of muscle tissue with lipids both in lean and obese PCOS either due to lipodystrophy or due to fat excess may be one of the accidental causes of IR [73]. Transforming growth factor-beta (TGFβ) signaling contributes to the remodeling of reproductive and hepatic tissues in women with PCOS. It is possible that these adverse effects including profibrotic changes of extracellular matrix influence insulin signaling in skeletal muscles [74, 75]. The most recent study by Stepto et al. tested the hypothesis that TGFβ superfamily ligands signaling pathways and tissue fibrosis are involved in PCOS-specific insulin resistance. These signaling defects are probably involved in PCOS ovulatory dysfunction too [76]. The results of this study showed reduced signaling in PCOS of the mechanistic target of rapamycin (mTOR). Notably, exercise augmented but did not completely rescue this signaling defect. Molecular tests showed that genes in the TGFβ signaling network were upregulated in skeletal muscle in the overweight women with PCOS but were unresponsive to exercise except for genes encoding lipid oxidation, collagen 1 and 3 [77]. Authors admit a limited number of patients and inability to

rule out the influence of other factors, such as HA, cardiometabolic fitness, and body composition. In *in vitro* study of TGFβ effects on myotubules suggest its indirect role in peripheral IR in PCOS [78]. Another study with *in vivo* and *in vitro* arms state that altered mitochondrial-associated gene expression in skeletal muscle in PCOS is not preserved in cultured myotubes, indicating that the *in vivo* extracellular milieu, rather than genetic factors, may drive this alteration [79]. Thus, molecular dysfunctions underlying peripheral resistance in women with PCOS in combination with the hormonal milieu need further investigation as they are likely to be the main cause of intrinsic IR and a perspective therapy target.

4. Specifics of androgen excess in NonObPCOS

HA is one of PCOS diagnostic criteria both by NIH, Rotterdam, and AE-PCOS criteria. Clinical HA implies mainly the presence of hirsutism. Biochemical HA is a stable elevation of circulating androgens over gender, age, and population-specific reference range. Free testosterone was traditionally considered the best maker of active androgen excess, but as its assessment with available indirect methods is not enough, an accurate estimated value of free androgen index (FAI) is recommended for clinical routine [1, 80]. In 2018, active androgens' precursors (dehydroepiandrosterone sulfate (DHEA-s) and A4) were recognized as useful markers of mild HA present in about 30% of PCOS and recommended for lab assessment in some cases [1]. Recent studies have demonstrated that 11-oxygenated androgens can be regarded as a marker of HA in PCOS [81]. Multiple bidirectional effects of HA with IR, OD, and adiposopathy in PCOS as well as their biological effects were consistently described in many reviews [16, 50, 82].

In this chapter, we address the proportional contribution and specific effects of androgens from different sources in NonObPCOS. HA in PCOS has complex nature involving ovarian, adrenal sources, peripheral tissue androgen synthesis, and conversion; elevated free T due to low sex-steroid binding globulin (SHBG). All these components are highly interconnected with IR, ovarian hormones, adipose tissue distribution, and function [3, 82]. The role of peripheral androgen convention is the least investigated aspect of HA in PCOS. But this specific aspect is important in terms of the disproportionate severity of clinical and biochemical HA often observed both in NonObPCOS and obese PCOS. In NonObPCOS, HA symptoms and biochemical HA are sometimes disproportionate to IR and OD that demands differentiation with secondary polycystic changes of ovarian morphology or investigating other than ovarian dominating androgen excess sources. Moreover, HA may have different metabolic sequelae depending on its origination. Contemporary methods of steroid metabolome assessment may open a new page in understanding the wholesome picture of androgen excess in PCOS.

The dominance of adrenal androgen excess was reported in older studies [83, 84]. In 2015, Moran et al. paper A4 and DHEA-s levels were significantly higher in nonobese than in obese PCOS patients. A significant correlation between luteinizing hormone (LH) and A4 in nonobese PCOS patients was observed. The frequency of hyperandrogenism by increased A4, and DHEA along with DHEAs was significantly higher in NonObPCOS compared with high-BMI PCOS patients [85]. In a 2015 review paper by M. Goodarzi, E. Carmina and R. Azziz analyzing the issue of adrenal androgen precursors' elevation etiology and role in PCOS conclude that inherited defects of steroidogenesis may be one of the causes and have to be further investigated; also there

is the intrinsic exaggerated activity of hypothalamic-pituitary-adrenal axis, while extra-adrenal factors, such as IR, play a limited role in the adrenal androgen precursors excess of PCOS [86].

A new study addressed specifically the issue of androgen excess sources in obese and NonObPCOS using liquid chromatography-tandem mass spectrometry and genetic tests. Its results showed increased DHEA-s, 17-hydroxyprogesterone(17-OHP), 17-hydroxypregnenolone, and estrone (E1) levels in NonObPCOS compared with both the lean controls and the obese PCOS patients, while lower FAI was found in the lean PCOS patients compared with the obese PCOS patients. The correlation analysis showed that FAI was positively correlated with BMI and HOMA-IR, which is in line with previous studies [34, 53, 87]. Enzyme activity evaluation showed that NonObPCOS had increased activity of cytochromes P450c17, P450aro, 3β-hydroxysteroid dehydrogenase type 2 (3βHSD2) and decreased activity of P450c21. Higher frequencies of CYP21A2- (encoding P450c21) c.552 C > G (p. D184E) in NonObPCOS were found compared with obese patients. The limitation of this study was that the gene sequencing was performed only for those with HA [88].

Active androgens and androgen precursors seem to have different effects on metabolism [89]. This is clearly demonstrated in a new cross-sectional study of 823 women with PCOS 76.2% with biochemical HA and 23.8% with normal androgen levels. Anthropometric indexes were used to assess metabolic risk characteristics. In normoandrogenemic PCOS, FAI predicted significant abnormality in the visceral adipose index (VAI) and dehydroepiandrosterone (DHEA) predicted against alteration in β-cell function. In HA PCOS, FAI predicted derangements in waist TG index and lipid accumulation products. DHEA weakly predicted against VAI, DHEA-s tended to predict against the abdominal obesity index [90].

Thus, existing studies show that NonObPCOS women have different profiles of androgen excess sources. Evaluation of the prevailing source of androgen excess might be a valuable component of metabolic risk estimation since some types of androgens seem to have a protective role. Also, more studies on steroidogenic enzymes function and alternative steroidogenic adrenal and peripheral tissue pathways are needed to have the whole picture of NonObPCOS pathogenesis.

5. Specifics of glucose and lipid metabolism in NonObPCOS

Inconsistencies in data on IR incidence in NonObPCOS can be explained by prevalent usage of indirect and not enough accurate methods of IR assessment. Indexes, such as HOMA-IR and others, have a good positive predictive value, but a poor negative predictive value [34, 91]. First studies proving the presence of hyperinsulinemia and IR in NonObPCOS with the use of gold standard method hyperinsulinemic euglycemic clamp were published in the nineties [92, 93]. The presence of unique disorder of insulin action was hypothesized. More recent studies with larger groups and more sophisticated methods supported these findings [94]. A study by Stepto et al. showed that the prevalence of IR in PCOS is 75% in NonObPCOS, 62% in overweight controls, and 95% in overweight PCOS [95]. In 2016, meta-analysis of premenopausal women diagnosed with PCOS compared with a control group for insulin sensitivity, measured by euglycaemic–hyperinsulinaemic clamp, NonObPCOS, and overweight PCOS compared with their respective controls had lower insulin sensitivity with large and very large magnitudes [96]. In a recent study by Tosi, evaluation of insulin action on glucose and lipid oxidation, nonoxidative glucose metabolism, and serum

FFA in different PCOS phenotypes was performed. Results of this study showed that irrespective of phenotype, PCOS women had impaired insulin-mediated substrate use influenced by T levels [97].

Thus, studies using the gold standard method of insulin sensitivity assessment demonstrate the presence of hyperinsulinemia and IR in NonObPCOS while estimated indexes are often not enough sensitive to detect mild IR in fasting state. At the same time, it is necessary to take into account some limitations of clamp tests apart from the technical complexity and high price. In the case of intravenous glucose administration, intestinal factors (glucagon-like peptide (GLP1), glucose-dependent insulinotropic peptide (GIP)) are not involved. Thus, being a gold standard for IR evaluation clamp tests detects glucose uptake in specific conditions that are quite different from physiological. At the same time, recent investigations in TDM pay much attention to the role of postprandial dysmetabolism including postprandial dysglycemia and dyslipidemia [98]. Studies on postprandial dysglycemia in NonObPCOS are very limited. One study with obese PCOS women showed that area under curve (AUC) for TG, insulin, and glucose were higher compared to obese controls while AUC for high-density lipoproteins (HDL) was lower after meal after adjustment for BMI and HOMA-IR [99]. In a study including also NonObPCOS HOMA-IR and AUC for glucose, TG, very low-density lipoproteins, and total cholesterol were higher in PCOS compared to BMI-matched controls [100]. Thus, clinical assessment of postprandial dysglycemia could be a valuable tool for glucose metabolism impairments early detection in NonObPCOS. Well known tool for this purpose is the oral glucose tolerance test with 75 g of glucose hardly can be done often. For this reason, emerging methods like self-monitoring blood glucose and continuous glucose monitoring as well as biomarkers are very much awaited to be approved for routine use in PCOS. Standardized methods of postprandial dyslipidemia are not yet available. In terms of postprandial dysglycemia data on patients eating habits are of great importance, especially on the frequency of food intake.

Described above altered body composition as well as AT and skeletal muscles physiology combined with HA results not only in IR but in high rates of dyslipidemia in NonObPCOS. In meta-analysis elevated prevalence of high-TG and low-HDL were shown NonObPCOS (for high-TG: OR 10.46; 95% CI 1.39–78.56; for low-HDL: OR 4.03; 95% CI 1.26–12.9) [15]. Thus, laboratory monitoring for dyslipidemia which by some authors is regarded as an IR marker is warranted for all NonObPCOS patients [101].

Liver is an active participant in glucose, lipid, steroid, and protein metabolism. NAFLD is a clinical disease characterized by the histologic finding of ≥5% macrovesicular steatosis of the hepatocytes in individuals with nonsignificant alcohol consumption or other known cause of chronic liver disease [102]. Traditionally NAFLD was attributed to overt diabetes and obesity. Studies on metabolic obesity in general and specifically on NonObPCOS changed this view. 2018 meta-analysis shows that compared to the control group, the risk of NAFLD in the PCOS group was higher (OR = 2.25, 95% CI = 1.95–2.60). When stratified by BMI frequency of NAFLD risk was significantly higher in both obese subjects (OR = 3.01, 95% CI = 1.88–4.82) and non-obese subjects (OR = 2.07, 95% CI = 1.12–3.85). In addition, PCOS patients with HA had a significantly higher risk of NAFLD, compared with controls (OR = 3.31, 95% CI = 2.58–4.24) [103]. Some studies do not demonstrate such a strong association with HA [104].

Overall pathogenesis of NAFLD includes not only IR but a complex of factors: altered energy balance, adipose tissue excess, hormonal changes, genetic factors

[105]. As the liver secretes proteins, metabolites, and hepatokines to influence metabolism in other tissues presence of NAFLD in PCOS exacerbates all major and minor pathological circuits of the syndrome. Most vivid example is low SSBG secreted by the liver leading to higher levels of free T and HA symptoms. Thus, it is logical to detect NAFLD in NonObPCOS regarding epidemiological data and close pathophysiological associations of NonObPCOS features and NAFLD. Though screening for NAFLD is very restricted by currant guidelines on this pathology [102]. Liver biopsy remains a gold standard for diagnosis of NAFLD, but diagnostics ultrasound and transient elastography proved to be effective for noninvasive diagnosis [106].

Thus, there are many unsolved clinical issues of detection crucial alterations in NonObPCOS like mild IR, postprandial dysglycemia, and NAFLD. But still regarding existing scientific data search of solutions of these issues are among primary goals on the way to more effective NonObPCOS management.

6. Management of NonObPCOS

There is not enough evidence to make specific prevention recommendations for NonObPCOS women since most of the studies on lifestyle modification included either overweight/obese or mixed populations. Taking into account all the above-mentioned data on specifics of NonObPCOS physiology it is reasonable to educate patients on the risks of early dyslipidemia, NAFLD, and metabolic syndrome. It is reasonable to monitor these conditions on a regular basis and to raise patient's awareness of the importance of healthy lifestyle especially eating behavior including food frequency, respect of circadian rhythms as well as food characteristics [107]. These recommendations remain actual for NonObPCOS women taking combined hormonal contraceptives. Until evidence-based specific dietary recommendations become available women with NonObPCOS can be recommended to keep Mediterranean diet principles as it proved to be protective from cardiometabolic risks in different populations including PCOS [108]. Also, control of fructose intake is highly recommended in view of NAFLD risks [109]. In the absence of definitive data on types of exercise favorable for muscle metabolism of NonObPCOS general recommendations from 2018 guidelines should be translated to every patient [1]. The arrival of new diagnostic methods for steroid metabolome, different metabolites (ceramides, bile acids, fatty acids, etc.), and gut microbiota assessment are promising in reaching targeted approaches for symptoms relief and MS prevention in NonObPCOS [110]. A number of pharmacological agents are promising for affecting main pathogenic mechanisms of NonObPCOS (insulin signaling defects, mitochondrial dysfunction, oxidative stress) and their consequences (IR, hyperinsulinemia, postprandial dysglycemia, ovarian dysfunction, dyslipidemia, NAFLD) including nutritional products and complex synthetic molecules. Among them are vitamin D, inositols, quercetin, resveratrol, L-carnitine, thiazolidinediones, GLP-1 receptor agonists, antihyperlipidemic drugs [111–119]. But all these groups of medications need an evaluation of their efficacy in properly designed clinical trials.

7. Conclusion

The prevalence of NonObPCOS is probably underscored. In the absence of high BMI unrecognized metabolic risks lead to their delayed diagnosis and interventions in

NonObPCOS. Existing data on NonObPCOS suggests that intrinsic alterations in adipose and muscle tissue function might be the starting point of key pathogenic factor – IR and consequent hormonal and metabolic derangements. There is a need for deeper investigation and improvement of diagnostic approaches to mild IR, steatohepatosis, and androgen excess sources for better management of NonObPCOS.

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Section 2

The Treatment of Polycystic Ovarian Syndrome

Chapter 6

Novel Methods in the Diagnosis of PCOS: The Role of 3D Ultrasonographic Modalities

Apostolos Ziogas, Emmanouil Xydias and Elias Tsakos

Abstract

Polycystic ovary syndrome (PCOS) is a common and complicated endocrine disorder, with its diagnosis based on clinical, laboratory and imaging criteria. The latter is usually assessed via two-dimensional ultrasound; however, the advent of three-dimensional ultrasound, along with three-dimensional power Doppler (3D-PD) could offer more accurate diagnoses and further our understanding of PCOS pathophysiology. Three-dimensional ultrasound (3D-US) has already been used successfully in many fields of gynecology. It offers improved image quality with stored data that can be processed either manually or automatically to assess many parameters useful in PCOS assessment, such as ovarian volume, number of follicles and vascular indices. The examination requires minimal time as data is assessed in post-processing, thus being more tolerable for the patient. 3D-US parameters are generally increased in PCOS patients when compared to controls and 2D measurements, with studies showing improved diagnostic performance, though that remains inconclusive. 3D transrectal ultrasound is more accurate in the diagnosis of virgin PCOS patients than the modalities currently available in that subgroup. Overall, though with some limitations, 3D-US is a promising diagnostic method in the assessment of PCOS which, regardless of diagnostic accuracy, can undoubtedly offer many practical advantages, more objective and reliable measurements, potentially improving PCOS diagnosis standardization.

Keywords: polycystic ovary syndrome (PCOS), 3D-transvaginal ultrasonography (3D-TVUS), 3D-power doppler angiography (3D-PDA), 3D-transrectal ultrasonography (3D-TRUS)

1. Introduction

Polycystic ovary syndrome (PCOS) is a complicated and heterogenous endocrine disorder affecting more than 10% of women worldwide and it is the most common endocrinopathy of women of reproductive age [1]. It is a syndrome with varied clinical manifestations and several degrees of severity. Some characteristics observed in PCOS patients include hyperandrogenemia, accompanied by acne and hirsutism, ovulatory dysfunction such as oligomenorrhea or amenorrhea, obesity, insulin resistance etc [1].

Diagnosis of PCOS was initially based on clinical characteristics alone, with three prevalent clinical features being agreed upon at the first international conference on PCOS [2, 3], namely:

- Chronic anovulation.
- Hyperandrogenism (evidently based on either clinical or laboratory findings).
- Absence of other endocrine disorders (i.e. adrenal hyperplasia, hyperprolactinemia, hyperthyroidism, hypothyroidism etc).

This definition and the diagnostic algorithm were lacking in several ways [3]. The associated clinical features necessary for diagnosis varied considerably in their clinical manifestation among patients, in particular menstrual instability, obesity and hirsutism and acne with the latter two being the manifestation of hyperandrogenism [4, 5]. Furthermore, no ultrasonographic evidence of PCOS was included in the diagnostic guidelines, although such evidence of PCOS was becoming more and more frequently included in the diagnostic workup of PCOS, with several centers, in fact, mandating it [6].

This led to a joint conference of the American Society for Reproductive Medicine and the European Society for Human Reproduction & Embryology in Rotterdam in 2003, where the previous diagnostic guidelines were revised [7]. The new Rotterdam criteria dictated that the diagnosis of PCOS must include at least two of the following:

- Chronic anovulation.
- Clinical or biochemical findings of hyperandrogenism.
- Clear PCOS findings on ultrasonographic scans.

With the revised criteria both hyperandrogenism and anovulation do not need to be present if ultrasound findings exist for the diagnosis of PCOS, thus including women that would elude diagnosis if the previous criteria were applied. The aforementioned ultrasound features necessary for PCOS diagnosis are the following [8]:

- Twelve or more follicles present.
- Follicle diameter 2–9 mm.
- Increased ovarian volume, more than 10 cm^3.
- Presence of the above features in at least one ovary.

The Rotterdam 2003 revised criteria constitute an important step in the standardization of diagnostic workup in PCOS, however, they do come with certain limitations. One of the most notable ones is the fact that ovarian volume measurements, collected based on data from 2D scans, mandate the use of a mathematical formula and therefore entail certain geometric assumptions and estimates [9]. A formula for a prolate ellipsoid (0.5 × length × width × thickness) is typically used, however, such calculations assume ovarian regularity, whereas PCOS ovaries have been repeatedly shown to be

more irregular than normal ones [10]. Another limitation of current diagnostic criteria is the lack of ovarian stromal volume and blood flow assessment. It has been shown that PCOS ovaries have increased stromal volume and blood flow [11, 12], two important parameters that could not only assist in the improvement of our understanding of the pathogenesis of the disease, but also serve as possible response predictors in the treatment of PCOS [13, 14]. However, neither of the two parameters are included in the 2003 guidelines, which could be partially attributed to the great degree of observer subjectivity in the description of stromal echogenicity, as well as to the technical difficulties in blood perfusion measurements using conventional Doppler ultrasound.

2. Three-dimensional ultrasound in gynecology

2.1 Technical aspects

Three-dimensional ultrasound (3D-US) as imaging technology was first developed in the 1980s and initially applied mostly in obstetrics for more accurate monitoring of fetal in utero development during pregnancy [15]. However, its success in that field led to research and trials for its potential application in gynecology as well.

Mirroring the application of two-dimensional ultrasound (2D-US), its more well-established and clinically applied counterpart, 3D-US is predominantly used transvaginally in the gynecological examination. The transducer is placed in close proximity to the target area and a complete 3D volume is acquired, which can be assessed either in real-time or digitally stored for later analysis. This option of data storage is particularly advantageous, as data acquisition via sweeping can be completed in seconds and thorough assessment at a later time significantly shortens the total examination time. This renders 3D-US a more tolerable and less time-consuming diagnostic modality overall.

The stored data can be displayed in several different ways, including display in three orthogonal planes, surface rendering, individual slice display (similar to conventional tomography) etc. This option for alternative displays can additionally provide detailed information about areas not previously accessible via conventional two-dimensional ultrasonographic display, namely, the coronal plane, which can significantly contribute to the diagnosis of uterine corner and adnexal pathology.

2.2 Application in benign gynecological disorders

3D-US over the years has been tested and applied in many benign gynecological disorders with varying levels of success, though on average, its performance is at least comparable to the conventional diagnostic methods and yielded results promising for its inclusion in the diagnostic work-up in clinical practice.

3D-US has been successfully applied in the diagnosis of congenital uterine abnormalities, with remarkable results, as studies have shown up to 100% sensitivity and specificity [16, 17]. Furthermore, research has proven the potential contribution of 3D-US to treatment optimization of uterine abnormalities as well, with 3D-US offering auxiliary visual guidance to the surgeon and improving the final surgical outcome [18]. Another application of 3D-US is in the evaluation of leiomyomas, offering the advantage of precise mapping of their location and clearer differentiation between intramural and submucosal variants when compared to the conventional method of assessment, namely 2D-US [19]. The 3D power Doppler modality is

beneficial in leiomyoma assessment as well, via the more precise evaluation of its vascularization. Therefore, more accurate selection and designation of patients as candidates for embolization treatment can be made [20]. The application of 3D-US in adenomyosis has been examined, with research showing encouraging results, as it facilitates superior visualization of the disrupted border between the endometrium and the basal endometrial layer [21, 22]. 3D-US has also been utilized in the assessment of intrauterine contraception device (IUD) malposition. It can clearly depict the device in its entirety and its position relative to the myometrium via the coronal view [23], whereas such images are far more challenging to obtain via conventional 2D-US. 3D-US also seems promising in pre-operational pelvic assessment in cases with deep pelvic endometriosis. Results are comparable to 2D-US and MRI in patients with intestinal loci of endometriosis and superior to the aforementioned imaging techniques in non-intestinal loci [24].

Apart from improving on currently available diagnostic techniques, 3D-US technology provides new, automated modalities as well. Such modalities mainly include being automated volume calculation, antral follicle counting and follicular growth monitoring, mainly utilized during IVF cycles. This technology has been shown to reduce overall cost, examination time and to deliver accurate and reproducible measurements as well [25–27].

2.3 Application in gynecological oncology

Regarding ovarian malignancies, 3D-US can accurately measure the volume of the mass, as well as visualize its internal structure, including wall irregularities, cystic elements, septae and so on [19], thus more accurately identifying suspicious masses [28]. In addition to 3D-US, 3D Doppler can offer precise information regarding mass vasculature, with increased mass perfusion and highly irregular vessel anatomy being indicative of possible malignancies [29]. In endometrial cancer, 3D-US can accurately measure endometrial volume, which is an important predictor of malignancy, as well as 3D Doppler vascular indices, however, more research is required to establish optimal cut-off values [30].

3. Three-dimensional ultrasound in PCOS

3.1 Technical aspects

As has been made evident so far, 3D-US has been successfully applied in the diagnostic work-up of many gynecological pathologies. Therefore, it was inevitable that similar research would be conducted on its application in PCOS assessment, particularly since the currently used technology does come with certain limitations as mentioned above.

Measurements and data acquisition methods vary between referral centers and studies, however, a similar procedure is followed. Measurements usually begin with a brief 2D-US assessment of the pelvis, followed by identification of the ovaries, with follicles larger than 10 mm and ovarian cysts being excluded. Subsequently, 3D mode is entered and the area of interest is defined. Subsequently, slow-sweeping at a 90° sweep angle or 30–45° angles is applied to ensure that the whole ovary is scanned [31, 32]. The resulting volumetric data is then stored for later evaluation. Compatible software, such as 4D view, allows for several calculations and measurements,

with techniques for ovarian volume, follicle count and ovarian stromal volume calculations.

Ovarian volume can be calculated by the rotational method, which in brief entails measurements at different rotational angles of the stored volume [31]. Follicle count can be facilitated by inversion mode, which entails setting a specific threshold that dictates which tissues are displayed. Therefore, it could be set to display liquid-filled, hypoechoic formations only, without the surrounding stroma, leading to easier and more accurate follicle counting. The same basic principle can be applied to display ovarian stromal volume or follicular volume and subsequently the voxels above or below the defined threshold can be automatically and accurately calculated to determine the OSV or the total follicular volume. This is known as semi-automatic measurement [31]. More recently, fully automated software such as Sono-AVC can automatically calculate the ovarian volume and follicle count, forgoing the traditional manual methods and providing more objective measurements with remarkable accuracy and reproducibility [32–34]. An example of automated follicle detection and the count is displayed in **Figures 1** and **2**.

3.2 3D-US parameters

3.2.1 Ovarian volume (OV)

OV is an important ultrasonographic parameter that has been included in the Rotterdam criteria and is typically increased in polycystic ovaries and PCOS when compared to controls. The same observations are made when OV is measured via 3D-US, however, 3D measurements have been proven more reliable than 2D ones [35],

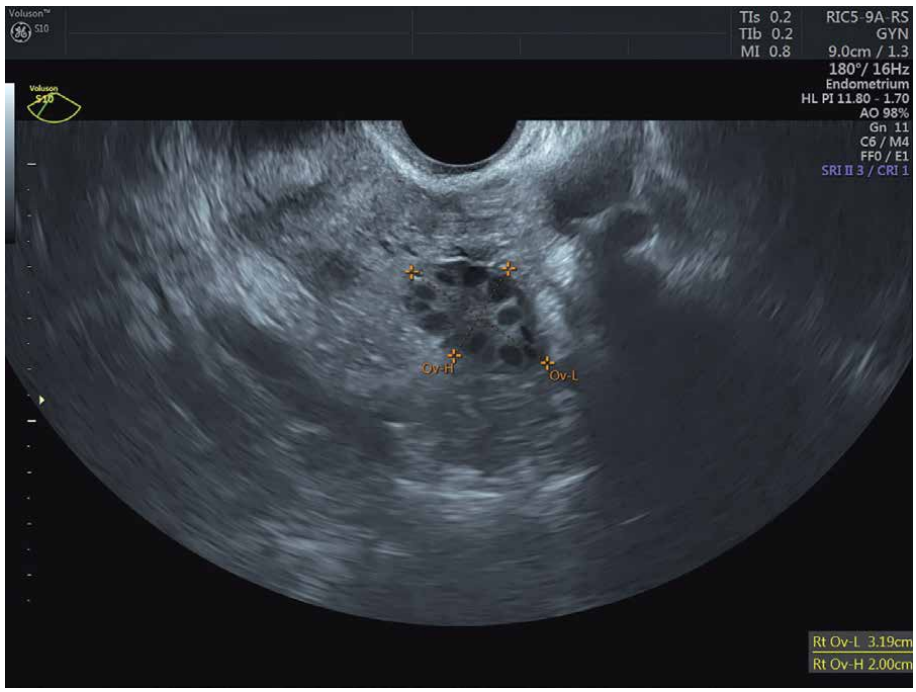

Figure 1.
2D slice of a stored 3D volume of a PCOS ovary.

Figure 2.
Automatic follicle detection and count via post-processing software.

Study	PCOS group					Control group				
	Num	**BMI**	**OV**	**OSV**	**AFC**	**Num**	**BMI**	**OV**	**OSV**	**AFC**
Lam [31]	40	27.35	12.56	10.79	16.3	42	24.1	5.66	4.69	5.5
Pascual [36]	38	23.3	13.21	N/A	22.5	45	21.3	6.65	N/A	7.4
Lam [37]	40	23.73	12.32	9.74	15	40	21.23	5.64	4.07	5.5
Alcázar [38]	42	23.5	11.2	N/A	22.5	38	23.1	5.6	N/A	7.4
Battaglia [32]	112	20.8	12.6	11	14.5	52	21.1	5.7	4.2	3.2
Sujata [39]	86	25.71	11.23	9.71	17	45	23.02	5.72	4.75	7

Abbreviations: Num: number of participants in the group, BMI: body mass index, OV: ovarian volume (cm^3), OSV: ovarian stromal volume (cm^3), FC: follicle count, N/A: not assessed.

Table 1.
Comparison of three main ultrasonographic parameters assessed by 3D-US in PCOS patients and controls.

as those necessitate certain mathematical assumptions. OV measurements in PCOS patients and healthy controls via 3D-US are presented in **Table 1**.

3.2.2 Follicle count (FC)

FC is critical in the ultrasonographic diagnosis of PCOS and is included in the Rotterdam criteria. FC can be referred to as antral follicle count, or follicle number per ovary, or even total follicle count in different studies, but practically they are

the tertiary follicles adjacent to a fluid-filled cavity, or antrum and can be visualized accurately via ultrasound if they measure more than 2 mm in diameter [33]. Typically and in fact by definition, PCOS patients and patients with polycystic ovarian morphology have increased FC compared to controls. Follicle counting by 3D-US can be more easily conducted compared to conventional 2D-US, like inversion, the model can be applied and seamlessly differentiate between liquid-filled cystic components and the surrounding stroma [3, 31]. Additionally, automated counting software offers a diagnostic alternative to manual counting, which while not conclusively proven to possess superior diagnostic accuracy, is reportedly less time-consuming [36].

Regarding quantitative data, Allemand et al. found that with 3D-US and by application of the subtractive method, mean FC in PCOS patients was 29.8 ± 11.5 and in controls, 9.5 ± 3.1 and the optimal cut-off for PCOS prediction was 20 or more follicles, with 70% sensitivity, 100% specificity and 0.987 AUC resulting in no false-positive diagnoses [40]. Their proposed threshold is higher than what is included in the Rotterdam criteria [7], which can be partially attributed to the use of 3D-US, as it has been shown to measure larger FC than 2D [41]. On that basis, they propose a possible revision of said criteria to include greater thresholds when 3D-US is applied. FC measurements in PCOS patients and healthy controls via 3D-US are presented in **Table 1**.

3.2.3 Ovarian stromal volume (OSV)

OSV has been considered an important ultrasonographic parameter in PCOS patients and is measured in many studies. Such measurements were traditionally conducted manually and showed that there is a statistically significant increase of stromal volume in PCOS patients compared to controls [11], perhaps indicating that hypertrophy of the thecal cells of the ovarian stromal is the main androgen-producing factor in PCOS, as has been hypothesized [31]. 3D-US allows for the calculation of OSV, that being either manual via the activation of inversion mode or automatic via thresholding, with the latter being less time-consuming than the aforementioned manual methods.

OSV has not been included as a parameter in the Rotterdam criteria, possibly due to concerns of subjectivity during measurements. 3D ultrasonographic calculation of OSV may present an opportunity to re-evaluate that fact, as OSV could prove to be very useful in clinical practice [37]. OSV measurements in PCOS patients and healthy controls via 3D-US are presented in **Table 1**.

3.2.4 Ovarian stromal volume to total ovarian volume ratio (OSV/OV)

OSV/OV is a proposed diagnostic parameter for the assessment of PCOS patients, based on the increase of stromal volume that has been observed in many studies. Battaglia et al. calculated this parameter in their study and concluded that it was the most accurate predictor of both hyperandrogenemia and hirsutism, with an AUC of 0.915 and 0.891, respectively when compared to every other ultrasonographic parameter assessed, such as OV, FC, 2D and 3D Doppler indices [32]. They also showed that an OSV/OV ratio equal to or greater than 0.84 was the optimal cut-off for the prediction of the aforementioned PCOS manifestations, with a sensitivity of 92% and a specificity of 91%. In addition, this parameter was more accurate if based on 3D-US measurements rather than 2D-US, which can be attributed to the visualization of the stroma of the whole ovary in 3D, compared to measurements conducted on a single 2D slice [32, 42].

3.2.5 Total follicular volume (TFV)

TFC is a parameter not used as regularly as the others in PCOS assessment, as its contribution is still in debate. Nardo et al. showed that it was in fact better correlated with PCOS laboratory findings compared to stromal volume and proposed that it was the increase of TFV that actually caused the increase in OV in PCOS patients rather than that of stromal volume [43]. This is in disagreement with several other studies showing that there is an increase in stromal volume and that it is an important predictor of PCOS, with TFV being lower in PCOS patients than in controls and being used mainly to calculate the OSV via subtraction from the total OV [32].

3.3 Comparison of 3D and 2D ultrasound

The comparative studies about 3D-US and 2D-US are not conclusive on one method's superiority over the other with regard to PCOS and polycystic ovarian morphology diagnosis.

On the one hand, some studies showed that the two methods showed no statistically significant difference between them as far as assessing the main ultrasonographic parameters, namely, FC and OV. Battaglia et al. found no significant difference between 3D and 2D-US parameters, however, they do acknowledge that 3D-US is a more appropriate method due to its reproducibility, it is requiring less mathematical assumptions, and its blood flow parameters assessed via 3D-Doppler [32]. As mentioned above, they also proposed the OSV/OV ratio as an important predictor, which was more accurate when based on 3D-US data. Similar conclusions were reached by Sujata et al. [39] as well as far as the comparison of 2D and 3D is concerned, with no significant difference between them is being made apparent.

Studies examining just the differences in FC between the two methods, outside of the PCOS setting also showed that 2D-US produced larger FCs. Deb et al. compared 2D estimations to 3D manual and automated estimations (via the SonoAVC software) of FC, with SonoAVC underestimating FC compared to the two other methods. However, 3D-US images were used for 2D estimates in that study, which might have led to the increase in FC that was observed. Moreover, a lower FC might be indicative of fewer double-counting incidents compared to manual measurements, thus in fact reflecting a more accurate FC. Regardless of FC, automated 3D-US FC was shown to possess greater inter-observer reproducibility than the other two methods [44]. In another comparative study by Deb et al. regarding 2D and 3D-US FC measurements in subfertile women, it was shown that 2D measurements of FC were significantly larger than 3D, but 3D FC semi-automated counting via SonoAVC was significantly faster, averaging approximately 130 s whereas manual counting via 2D-US lasted for an average of 324 s [45].

On the other hand, Nylander et al. concluded that 3D-US was more accurate as far as OV was concerned compared to 2D, as the 3D estimates were in closer agreement with MRI measurements. In their study, 2D measurements of ovarian volume were 14.9% smaller than 3D-US measurements and 11.6% smaller than MRI, which is in agreement with one previous study comparing 2D to MRI and other studies comparing it to volume measurements of anatomical specimens [46]. This observation is attributed to the assumption of a regular ovoid or ellipsoid shape of the ovary and the use of mathematical formulas in the calculation of OV in 2D-US, whereas 3D-US, MRI and anatomical measurements outline the ovary contours and thus result in more

precise measurements [46]. Regarding FC, the research team found that 2D estimates were 18% smaller than those of 3D-US and 16% smaller than those of MRI, suggesting that 3D-US more accurately counts antral follicles than 2D-US.

Overall, the currently available bibliography is still conflicted on which of the two methods provides the most accurate measurements of ultrasonographic parameters, however, what is undisputed is the speed and reproducibility of 3D-US measurements, which is superior to 2D.

4. Three-dimensional power Doppler

4.1 Technical aspects

Three-dimensional power Doppler (3D-PD) allows for vascularization and blood perfusion assessment via histogram analysis and has been shown to provide more data than frequency-based Doppler ultrasound, especially in low-velocity flow and when flow alterations take place [47, 48]. It has also been considered a means of objective assessment of vascularization and blood flow, contrary to 2D modalities which examine only specific blood vessels in a single slice and depend on the detection of the most representative image of the examined pathology [48]. With regard to ovarian pathologies, 3D-PD via scanning the organ in its entirety could offer very representative data of the vascularization and perfusion status of the whole ovary and perhaps applied in clinical practice.

The data acquisition procedure closely resembles 3D-US acquisition of 3D volume data, with the notable difference of specific Doppler settings being activated to capture relevant data. Afterward, the acquired information is stored digitally and via post-processing software, such as VOCAL or 4D-view, computer algorithms create a histogram of voxel data and calculate vascular indices. The indices most commonly assessed are the vascularization index (VI), the flow index (FI) and the vascularization and flow index (VFI), as described by Pairleitner et al. [48].

4.2 3D-PD parameters

4.2.1 Vascularization index (VI)

VI is the proportion of the scanned volume that emits a flow signal compared to the rest of the organ. In practicality it is the number of colored voxels (representing areas that flow was detected) and expressed as a percentage of the complete volume of the ovary, thus reflecting the blood vessel density in the scanned volume. It could be applied in the diagnosis of pathologies where vascularization either increases or decreases, without changes in the blood flow necessarily, as VI provides no information on the blood flow itself or its intensity [32, 48].

4.2.2 Flow index (FI)

FI is an average of the signal intensity of the blood flow detected in the scanned volume. In practicality, the software calculates the mean color value of all the colored vessels, representing the average intensity of the blood flow in the scanned volume, which could be used in pathologies where there are changes in blood flow but not in the anatomy of blood vessels or vascularization [32, 48].

4.2.3 Vascularization-flow index (VFI)

Finally, VFI is the combination of the information provided by the other two indices, as practicality is the product of VI and FI. It could be applied to identify pathologies on the spectrum of low vascularization and decreased blood flow on the one extreme and increased vascularization and blood flow on the other [32, 48].

4.2.4 Mean grayness (MG)

Mean grayness is not a vascular index, as it assesses the mean signal intensity of the gray voxels, meaning areas without detectable flow. It is a more objective representation of the tissue echogenicity which is traditionally assessed subjectively via 2D-US, as it is calculated by algorithm based on histogram data. Despite it not being a vascularity index, in most studies it is assessed along with the other three 3D-PD parameters, therefore, data on it will be presented along with the other three in this chapter as well [32].

4.3 Study results on 3D-PD parameters

There have been numerous studies conducted on PCOS patients that used 3D-PD and calculated mean values for both PCOS patients and controls. This data is summarized in **Table 2**. There is significant variation regarding the values acquired among the studies. This could be attributed to differences in study design and protocol (definition of PCOS, time of ultrasonographic data acquisition relative to menstrual cycle), the technology used (different devices, heterogenous settings) and disparities in demographical characteristics of the participants (age, BMI, clinical manifestations etc).

In general, there is still a lack of consensus on whether these parameters can be utilized in PCOS assessment, with many studies showing that some or all indices were significantly increased in PCOS patients, whereas others showed no statistical difference between the values at all. Data on the statistical significance of the differences of several parameters between the PCOS group and the control group are presented in **Table 3**.

Study	PCOS group					Control group				
	N	VI	FI	VFI	MG	N	VI	FI	VFI	MG
Järvelä [49]	14	5.3	44	2.4	44.5	28	6.1	43.1	2.7	45.7
Pan [12]	25	3.99	50.26	2.1	N/A	54	1.44	44.44	0.8	N/A
Lam [31]	40	3.85	33.54	1.27	32.4	40	2.79	31.79	0.85	30.4
Lam [37]	40	2.56	30.19	0.82	22.4	40	2.41	29.36	0.73	23.3
Mala [47]	25	6.07	20.97	2.39	N/A	25	1.87	19.46	1.16	N/A
Battaglia [32]	112	4.2	35.5	2.3	30.9	52	1.7	27.1	0.8	18.6
Sujata [39]	86	10.7	16.84	1.79	N/A	45	10.0	16.35	2.17	N/A
Garg [50]	30	7.26	28.23	2.15	N/A	30	0.88	16.61	0.16	N/A

N: number of participants, VI: vascularization index (%), FI: flow index (0–100), VFI: vascularization flow index (0–100), MG: mean grayness (0–100), N/A: not assessed.

Table 2.
3D-PD parameter values in PCOS patients and controls.

Study	BMI	Day	OV	FC	VI	FI	VFI	MG	OPI	ORI
Järvelä* [49]	N/A	8–16	↑	M/A	ND	ND	ND	ND	N/A	N/A
Pan [12]	↑	2–3	↑	N/A	↑	↑	↑	N/A	N/A	N/A
Lam [31]	↑	3–5	↑	↑	↑	ND	↑	ND	ND	ND
Lam [37]	↑	3–5	↑	↑	ND	ND	ND	ND	ND	ND
Mala [47]	↑	2–5	↑	↑	↑	ND	↑	N/A	↑	↑
Battaglia [32]	ND	3–5	↑	↑	↑	↑	↑	↑	↓	N/A
Nylander [46]	ND	N/A	↑	↑	ND	ND	ND	N/A	N/A	N/A
Garg [50]	↑	2	↑	↑	↑	↑	↑	N/A	N/A	N/A

*BMI: body mass index, Day: day of the menstrual cycle that 3D-PD measurements were taken (note: in cases with amenorrhea, the days are counted after withdrawal bleeding induced via progesterone administration for a week), OV: ovarian volume (cm3), FC: follicle count, VI: vascularization index (%), FI: flow index (0–100), VFI: vascularization flow index (0–100), MG: mean grayness (0–100), OPI: ovarian pulsatility index, ORI: ovarian resistance index, ↑: significant increase in PCOS group, ↓: significant decrease in PCOS group, ND: no significant difference between the two groups, N/A: not assessed.*2D-PD parameters (OPI, ORI) were measured on uterine arteries.*

Table 3.
Several parameters of the PCOS group and their difference in comparison to control group measurements.

From the assessed parameters, VI and VFI appear to be the more reliable of the four parameters, as they are significantly elevated in every study that 3D-PD parameters significantly differ between the PCOS and control groups. FI and MG are not shown to be as reliable relative to the other two, as they do not differ between the two groups in two studies, whereas VI and VFI are increased [31, 47]. No difference or effect on these results was noted based on differences in age, BMI or day of the cycle when the 3D-DP scan was performed.

Some of the included studies compared to the traditional way of ultrasonographic assessment of vascularity and blood flow, namely 2D-PD with the most frequently used parameters being pulsatility and resistance indices of the ovarian vessels to the 3D-PD parameters. Though data on this comparison is only available from four studies, it is inconclusive, as in three of the studies 2D-PD parameters are statistically different between the PCOS and control groups and in one, no statistically significant difference between the two is evident, with the 3D-PD parameters following the same trends in these studies as well. Lam et al. note that is based only on 2D-PD measurements, no difference between the PCOS patients and healthy participants would be noted, whereas that distinction was made apparent when 3D-PD was applied [31].

As far as cut-offs and reference values are concerned, from the studies that did find a significant difference, only Battaglia et al. attempted to create ROC curves and calculate optimal cut-off values, however, since the ROC curve was not statistically significant, no such values were obtained. More research is required, mainly to confirm the significance of 3D-PD measurements, as in half the studies no significant differences were noted and establish optimal cut-off values that could herald the application of 3D-PD in clinical practice as an objective means of vascularization and blood flow assessment in PCOS.

5. Three-dimensional transrectal ultrasound (3D-TRUS)

PCOS generally manifests during adolescence, in young and usually virgin women. In such patients, the so far described transvaginal ultrasonographic assessment with

its remarkable diagnostic accuracy is not recommended. Therefore, the transabdominal and transrectal approaches are considered viable alternatives, with the latter seeming more promising, as it is frequently difficult to obtain high-quality images via TA-US [51].

The advent of 3D-US technology marks a significant advance in that field, as 3D-TRUS could replace it in the cases that transvaginal cannot be applied, with hopefully similar results. Sun et al. attempted to evaluate 3D-TRUS' diagnostic accuracy in such a population, namely virgin PCOS patients. A total of 45 virgin patients with PCOS, aged 15–25 presenting with the classic PCOS clinical manifestations were enrolled in their study. In addition, 30 patients with only the ultrasonographic findings of polycystic ovarian morphology and no clinical symptoms, along with 25 healthy volunteers were enrolled as well. All patients received 2D-TAUS and 3D-TRUS and several 3D parameters were assessed.

The results were very encouraging, as 3D-TRUS allowed for improved detection of PCOS, in fact even surpassing transvaginal sonography's accuracy, with the most accurate parameter being the stromal area to total area ratio. Though very encouraging for 3D-US application in this specific subgroup of young patients, whose family planning can be severely impacted by PCOS, the results of this study should be verified by future studies on the subject, as the authors stress [51].

6. Limitations of 3D-US

As with every other diagnostic method, 3D-US is by all means not without some limitations which should be mentioned.

For 3D-US high-quality image acquisition, typically the probes used are larger than the corresponding 2D probes, although not by much. Thus, in theory, this could render the examination less tolerable by the patients, especially in transvaginal or transrectal ultrasounds. However, this in practice is balanced by the shorter examination time, as mentioned above and as 3D technology constantly evolves, it is very likely that such concerns about the transducer size will be eliminated [52].

Another consideration is data storage, as 3D-US stores data regarding the whole volume of the target and not just slice as its 2D counterpart. Therefore more space is required to store patient data, with said requirements likely to further increase, as technology improves and image quality improves exponentially. However, this is offset by the synchronous progress of digital media as well, with digital storage becoming more and more affordable and health centers using servers thus rendering physical storage media, such as DVDs and USBs obsolete [52].

3D-US remains a costly method to this day, with the latest equipment usually being unaffordable by most centers. Apart from the physical devices and peripheral attachments, the cost of software is also a major consideration, as the more advanced modalities that facilitate automatic follicle counting and volume measurement are an additional cost for potential buyers. However, as technology progresses, 3D-US equipment will undoubtedly become more and more affordable, particularly by centers and individuals specialized in PCOS and other fields where it can be applied.

Another easily overlooked limitation is the need for 3D-US operator additional training. Despite the many apparent similarities with the more established 2D-US, special training is required to obtain high-quality images as well as to process the acquired data after the examination. Many inexperienced operators face orientation problems during post-processing and viewing, as the improved space awareness combined with

an initial lack of correct orientation determination during the examination can lead to the false perception of the stored volume and false assumptions [52].

Finally, like every other imaging technique, 3D-US can produce artifacts, some that are similar to 2D and others limited to themselves due to the acquisition process, the rendering and the post-processing. It is more usual in 3D-US for motion artifacts to be produced, as the whole organ must be scanned and not every patient can stay still throughout the examination. Therefore, training on data acquisition and correct post-processing is required to reduce the number of artifacts that may be introduced to the images, as well as training on artifact recognition, as misinterpretation of them can lead to inaccurate diagnoses.

7. Conclusion

PCOS is a common endocrine disorder affecting many women worldwide, with varying clinical manifestations. Diagnosis is mainly based on 2D-US, however, the relative advent of 3D-US technology offers a promising alternative. 3D-US entails the acquisition of the complete 3D volume of the region of interest, along with vascularization data if Doppler mode is applied. This process is quick and thus more tolerable for the patients and provides vastly more information than 2D real-time assessment. The stored data can be evaluated at any time by many examiners and the measurements of OV, follicle count and VI, FI, VFI and MG, especially if automatically calculated are more objective and with significantly better inter-observer reproducibility of results.

Data on actual diagnostic performance in comparison to the currently available technology is still lacking and inconclusive, with some studies showing no difference and others indicating that 3D-US more accurately visualizes the underlying ovarian morphology and thus offers a more accurate diagnosis. The bibliography on 3D-PD ultrasound is more conflicted, as many studies did not manage to show statistically significant differences in PCOS patients' Doppler parameters in comparison with the control group. However, some studies actually did show a significant difference and in fact propose that 3D-PD offers a more objective and accurate assessment of the vascularization and blood flow of the ovary, as it visualizes the whole organ and its parameters are calculated based on histogram analysis and not the operator's observations, thus being more objective. 3D-TRUS is shown to be a very promising alternative to the traditional transvaginal approach in virgin patients, with remarkable results.

It is made apparent that more research is required to further assess the diagnostic accuracy and usefulness of 3D-US in PCOS assessment, especially as far as 3D-PD is concerned, as it shows much promise and could potentially lead to the inclusion of objective diagnostic criteria in the guidelines if sufficient evidence is found. In addition, new reference values and cut-offs need to be established, again especially in 3D-PD, as the current bibliography is still lacking in that regard.

Conflict of interest

The authors have no conflict of interest to declare.

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Chapter 7

The Novelty of miRNAs as a Clinical Biomarker for the Management of PCOS

Rana Alhamdan and Juan Hernandez-Medrano

Abstract

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects around 5–10% of women of reproductive age. The aetiology of PCOS is not fully understood with various genetics, iatrogenic (e.g. chemotherapy) and environmental factors have been proposed. microRNAs (miRNAs) are small non-coding single-stranded RNAs which are known to act as a regulator to gene expression at the post-transcriptional levels. Altered expression of miRNAs has been linked to several disorders including infertility. Recent reports demonstrated the expression of differential levels of miRNAs in the serum, ovarian follicular cells and follicular fluid of PCOS patients when compared with healthy women. Therefore, miRNAs may play important role in the pathogenesis of PCOS. The aim of this chapter is to summarise the current understanding pertaining to miRNAs and PCOS and to expedite its possible role in the diagnosis and management of this disorder.

Keywords: PCOS, miRNA, follicular fluid

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common multifactorial endocrinopathy female disorder. It affects approximately 5–10% of women in their reproductive age [1–3]. It is characterised by oligoanovulatory ovarian dysfunction, polycystic ovarian morphology and clinical and biochemical hyperandrogenism (HR). In addition, other factors such as endocrine dysfunctions, suboptimal follicular environment and oocyte competence make it a leading cause of female infertility. PCOS is also an important risk factor for metabolic disorders such as obesity, hyperlipidaemia and insulin resistance (IR), leading to type-2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and endometrial cancer and other diseases [4, 5]. To date, the aetiology of PCOS remains elusive; however, environmental and genetic variations are proposed as a potential contributing factor. Although recent pedigree and genome-wide association studies have revealed apparent interrelation of several genes with PCOS, none have shown direct cause to the occurrence of the syndrome [6]. Thus, it has been postulated that their effect might be apparent in a dose-dependent or synergistic fashion rather than being a one or none effect [6]. Recent reports have suggested miRNAs' involvement in PCOS incidence and development, thanks to the differential expression in patients with this disorder and healthy fertile patients [1, 7].

2. miRNAs as a potential novel clinical biomarker for PCOS

miRNAs are a new class of endogenously produced short non-coding single-strand RNAs with 20–25 nucleotides [1, 4, 8]. miRNAs are first transcribed from the genome as a primary miRNA (pri-miRNA) by RNA polymerases to form a hairpin like structure. For the miRNA to exert its biological function, it cleaves into a precursor miRNA (pre-miRNA) by a nuclear protein complex containing a Dorsha enzyme. These small transcripts (60–110 nt) can then leave the nucleus to the cytoplasm and subsequently processed further by the Dicer enzyme and another protein complex into the mature form of the miRNA. The mature form of the miRNA can regulate gene expression post-transcriptionally via binding to the 3'untranslated region (UTR) of the target mRNA, thus preventing their translation and/or causing their destabilisation of molecule [1, 9]. miRNAs have been shown to be widely expressed throughout the body, including organs such as muscles, adipose tissue and ovaries, at the intra- and extracellular levels [1, 4]. Recently, miRNAs have been isolated from extracellular fluids with variable expression profile depending on the fluid [1]; urine [10], saliva, plasma/serum [9] and follicular fluid [11]. Furthermore, miRNAs can be encapsulated in microvesicles [12] which are extracellular hetergeneous membraneous vesicles (EVs) originating from the cells. The smallest subpopulation of which are called exosomes, and they are known to act as a mediator to transport and release miRNAs between target cells [12]. It has been reported that miRNAs are more stable in exosomes with greater gene regulatory activity [13]. miRNA's functions become feasible through participation in processes involved in biological growth, development and disease state. The main impact of miRNAs has been demonstrated during cellular proliferation, differentiation, metabolism, and apoptosis with regulatory roles on RNA processing and transcription, chromatin structure and chromosome segregation [14]. In humans, it has been reported that around 60% of proteins-coding genes serve as target sites of miRNA [14, 15]. miRNAs can also act in an epigenetic manner to regulate the amplification and inhibition of miRNA signals through the feedback mechanism which may lead to a significant modification expression that contributes to different pathological conditions [4]. Moreover, miRNAs have been shown to play an important role in ovarian physiology and pathology such as primordial follicle activation and development, oocyte maturation, ovulation, ovarian cancer and endometriosis [4, 14]. Growing evidence demonstrates a differential expression of miRNAs in patients with and without PCOS [1], potential diagnostic and therapeutic markers for PCOS. Nevertheless, considering the large degree of heterogeneity of PCOS and the complexity of miRNAs regulatory actions, to our knowledge to date is still preliminary. Understanding the role of miRNAs in PCOS pathogenesis is crucial for possible alternative management and treatment approaches to this syndrome.

To this end, the aim of this chapter is to summarise and discuss the current knowledge regarding the possible interplay between miRNAs and PCOS and to establish the potential clinical role of some miRNAs that may offer a novel insight for the management and treatment of the syndrome.

2.1 Possible role of miRNAs in ovarian dysfunction in PCOS

Several studies and theories have been proposed in an endeavour to explore the possible cause of the altered follicle growth and development, large number of small and generally immature follicles as well as anovulation associated with PCOS. Hormonal-induced alterations to granulosa cells (GCs) appearance and function, as

well as steroidogenesis abnormalities by the theca cells (TC), have also been reported (**Figure 1**). However, the mechanisms, chronology and the relative criticality in the cascade of events leading to PCOS are still unestablished. Since PCOS is largely influenced by environmental and genetic modifications including miRNAs [16], and their epigenetics alteration can modulate gene expression at the cellular levels, thus miRNAs' profiles in the ovarian compartments in PCOS have been studied in several animal and human models and are discussed further in the section below.

2.1.1 miRNAs in granulosa cells (GCs) and PCOS

During follicle development, GCs govern oocyte growth by modulating nutrient availability and activity of regulatory molecules. GCs have been identified as the primary site of endocrine signalling and oestrogen synthesis [17]. It has been demonstrated that GC may contribute to the abnormal folliculogenesis in PCOS patients [18]. These abnormalities have been further defined by the proliferation inhibition and increased rate of GCs death, thus supporting the link between the functional disorder of GCs and the disease nature of the PCOS [18].

A vast array of miRNAs has been shown to be differentially expressed among various size follicles. They have been also indicated to modulate GCs apoptosis and proliferation [19]. miR-1275, a regulator of GC death, has been reported to upregulate during follicular atresia and induce early porcine GCs apoptosis [20]. miR-23a and miR-27a have been shown to stimulate GC apoptosis, whereas miR-93 and Let-7 family of miRNAs act to promote proliferation [4, 17]. Moreover, Let-7 family of miRNAs has also been reported to induce GC apoptosis via the inhibition of mitogenic activated protein kinase 1 (MAP3K1). Interestingly, let-7c, 23a and 27a were all shown

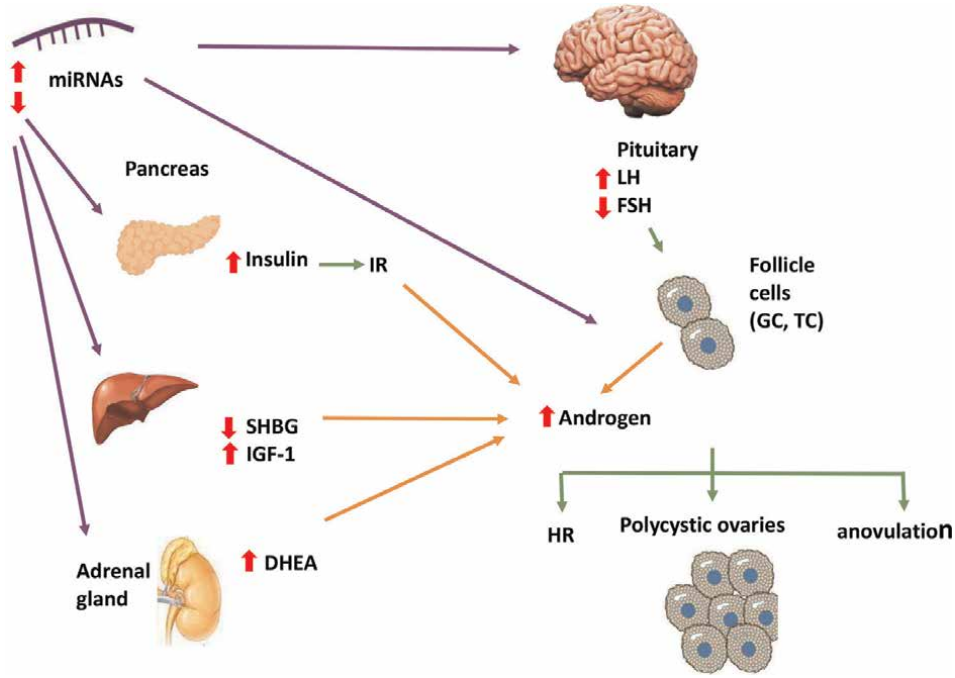

Figure 1.
The complexity of miRNAs interactions with multiple organs leading to PCOS.

to be highly expressed in patients with premature ovarian failure when compared with a healthy control patient [21]. Furthermore, a high expression of miR-93 [22] and lower miR-23a have been identified in the GCs from PCOS patients. They indicated that miR-23a induces cell cycle arrest via the inhibition of FGD4 signalling [23]. Another group has also demonstrated a significantly lower expression of miR-126-5p and miR-29a in the GCs of PCOS patients when compared with healthy individuals, which were indicated to induce GC apoptosis in PCOS [24]. A recent study proposed that the high expression profile of miR-141 and miR-200 in PCOS may target the Wnt and PI3K signalling pathway to inhibit GC proliferation [25]. Moreover, miR-3940-5P, miR-486-5P, miR-206 and miR-204 are known to modulate ovarian GC proliferation and apoptosis [26, 27]. In PCOs, miR-3940-5p was reported to be markedly upregulated; however, low expression of miR-206, miR-204 and miR-486-5p has been indicated in the GC of PCOS when compared with normal controls [19, 26]. One questionable miRNA, which has been known to be involved in the proliferation and apoptosis of GC, is miR-485-5P. This miRNA has been reported contradictory in PCOS, with one report demonstrating its upregulation [18], and the other showed a lower expression of miR-485-5P in the GC of PCOS [28].

Furthermore, the dysregulation of miRNAs in GC can be a cause of oestrogen difeiciency which is known as a main characteristic of PCOS. Zhang et al. [29] reported a significantly lower expression of miR-320a in the cumulus cells (CCs) of PCOS, which served via 320a/RUNX2/CYP11A1 (CYP19A1) cascade to induce oestrogen deficiency. miR-182 and miR-15a are known as an essential regulator of GC proliferation and apoptosis as well as steroidogenesis. These miRNAs were found to markedly decreased in the GC of PCOS patients [4].

Studies on miRNAs expression in GC and its relation to PCOS are intensive and generally linked to the increased GC proliferation and apoptosis rates in GCs. Although it may sound contradicting, but it does make sense when thinking about the progression features of the disease in relation to follicular development. The increased proliferation rate of GCs can contribute to a more follicles progressing to the primary stage, which is reflected in the polycystic countenance of PCOS [30]. Whereas the increased apoptosis rate is indicated by the anovulatory feature of the disease [4, 31].

Overall, to date, all reports indicate that GCs miRNAs are clearly involved in the regulation of folliculogenesis and steroidogenesis, and any dysregulation or alteration may subsequently contribute to the pathogenicity of PCOS.

2.1.2 miRNAs in theca cells (TCs) and PCOS

Theca cells (TCs) are the primary site of androgen synthesis in the ovaries. Under the influence of LH, theca cells express the mRNA of the three important steroidogenic enzymes CYP11A, CYP17 and 3ß-HSD, which are involved in the androgen *de novo* synthesis pathway [32, 33]. Androgen excess is one of the most important key features that is used to diagnose PCOS [14, 34]. It can negatively impact ovarian follicle growth and maturation and therefore female infertility. It has been reported that the increased expression or activity of CYP17 and P450scc enzyme is associated with the abnormal high androgen production in the theca cells [2, 35, 36]. Another key androgen producing-related gene GATA6 and an insulin receptor gene IRS-2 were demonstrated to be markedly upregulated in the theca cells of PCOS patients. Lin et al. [2] documented that miR-92a and miR-92b are involved in the regulation of GATA6 and IRS-2, and their expressions were significantly lower in theca tissues of women with PCOS. The results of miRNA microarray analysis have demonstrated that

miR-200a, miR-200c, miR-141 and miR-502-3p were significantly increased in the theca interna in women with PCOS [14].

Limited data are available on the theca cells miRNAs in PCOS; however, it indicates that it may play a pivotal role in the dysregulation of androgen synthesis pathways in women with PCOS.

2.1.3 miRNA in follicular fluid and PCOS

Follicular fluid (FF) provides a perfect microenvironment for oocyte development and maturation. It allows for an efficient cross talk between the blood, granulosa and TCs [9]. FF comprises various hormones, multiple proteins, metabolites, anti-apoptotic factors and regulatory nucleotides including miRNAs [13]. Therefore, FF composition reflects the secretory activity of oocyte, GC and TCs. In addition, it may serve as a relatively easy and less invasive method for the collection and the analysis of miRNA during oocytes retrieval for assisted reproduction [4].

miRNAs have been reported abundant in FF, and some researchers focused on investigating their association to PCOS [1, 13]. A recent study found 29 differentially expressed miRNAs in the FF of subjects with and without PCOS, with 12 involved in reproductive pathways [37]. Of these, miR-382-5p was found to positively associate with free androgen index (FAI) and age, miR-199b-5p allied with AMH, and miR-127-3p was linked to insulin resistance [37]. A study by Roth et al. [38] reported a significantly increased expression of miR-9, miR-18b, miR-32, miR-34c and miR-135a in the FF of women with PCOS. In addition, the study demonstrated that synaptogamin 1, insulin receptor 2 and interleukin 8 were the target genes for these miRNAs [38]. The study by Scalici et al. showed an increased expression of miR-30a and a significantly decreased levels of let7b and miR-140 in the FF of PCOS individuals. FOXL-2, essential gene for ovarian development, was identified as the target gene for miR-30a, and the inhibition of this miRNA in mouse resulted into disrupted GC morphology and androgen production by the TCs [39]. Furthermore, two members of the TGF-ß family, Smad 2/3 and activin receptor I, were possible target genes for let-7b. The dysregulation of TGF-ß was reported to be a potential cause of PCOS [1, 40]. Scalici et al. [39] also concluded that the combination of Let-7b, miR-30a and miR-140 can be used as a possible novel biomarker, with a sensitivity of 70% and specificity of 80%, to distinguish between normal controls and PCOS. miR-93 has been described as a novel diagnostic marker for PCOS due to the significantly consistent increased expression when compared with healthy individuals [41]. Moreover, a study by Lin et al. reported a significant downregulation of miR-92a and miR-92b in PCOS [2].

Studies focusing on miRNAs' involvement in androgen metabolism and PCOS have identified a specific group of miRNAs in the FF of PCOS patients; however, there is no specific consensus on the effect of these miRNAs in steroidogenesis in PCOS patients. Of these miRNAs, miR-151 was shown to negatively associate with free testosterone; while miR-29a and miR-518 were positively correlated to testosterone; miR-155, miR-9 and miR-18b inhibited testosterone and miR-146a, miR-132 and miR-135 inhibited both testosterone and progesterone secretion [42]. Other contradicting miRNAs, miR-132 and miR-320, which are known to be involved in oestradiol release, showed significant lower expression in PCOS patients [43], while another author reported increased expression of miR-320 in PCOS individuals [39, 44]. A third report demonstrated no change in 320a expression in PCOS subjects when compared with healthy women [3, 38]. miR-518-3p was shown to highly expressed in androgenic

PCOS phenotype. Further analysis demonstrated the reduction in miR-24-3p, miR-29a, miR-151-3p and miR-574-3p levels in PCOS subjects when compared with healthy controls [3].

All above, data indicates that miRNAs in PCOS can serve as a potential novel biomarker for the diagnosis and maybe further for the classification of different PCOS phenotypes. Furthermore, it may provide insights into the molecular changes related to the cells from which the fluid is derived from and thus support therapeutic decisions. Ovarian cells and circulating miRNAs that have been proposed to be dysregulated and involved in the pathogenicity of PCOS are summarised in the **Table 1**.

2.2 miRNAs altered androgenic and metabolic consequences of the PCOS

HA and metabolic disorders are inevitably associated in women with PCOS. It is always manifested by hyperandrogenism (HA), insulin resistance (IR) and compensatory hyperinsulinemia, which is known as an essential contributor to the pathogenicity of PCOS. Therefore, the mechanism behind this connection could shed lights on key markers in the diagnosis of PCOS.

2.2.1 miRNA and androgens dysregulation in PCOS

HA is a common characteristic of PCOS that is detected and used for diagnosis in both serum and ovarian compartment. The source of androgen excess is not exclusively resolved, it might be due to increased steroidogenic enzyme activity, increased androgen synthesis by the TCs as a response to LH overstimulation, androgen receptor (AR) defects at the target organs level, cortisol metabolism defects and/or increased adrenal gland androgen production [3, 4, 45]. Evidence indicates that testosterone, androstenedione (A4) and dihydrotestosterone (DHT) are all involved in the pathogenicity of PCOS. In normal subjects, a major fraction of free testosterone is bound to sex hormone binding globulin (SHBG) and albumin. In women with PCOS, SHBG levels are decreased resulting into an increased level of bioavailable testosterone [4]. Furthermore, increased expression of AR has been reported in patients with PCOS [46].

The complexity of abnormal sex hormone production makes it difficult to define the specific miRNAs involved in this process. Several studies have highlighted the role played by miRNAs in androgens and steroid synthesis in the ovarian cells and body fluids. miR-592 was found to positively correlate with LH/chorionic gonadotropin receptor (LH/CGH), a key factor in the mechanism involved in HA in PCOS [47]. A recent study showed a negative association between serum testosterone and miR-146a, an miRNA that has been found to inhibit the secretory activity of steroid hormones [1, 48]. Oestradiol was noted to positively linked with miR-222, miR-132, miR-320 and miR-520-3p, whereas a negative relationship has been observed between progesterone concentration and miR-193b, miR-24 and miR483-5p [43, 49]. Furthermore, miR-320, miR-518, miR107 and miR-29a were also found to positively correlated with high levels of serum testosterone [4, 9]. On the other hand, expression profile of miR-151 was found to negatively relate to serum testosterone [9]. Another study demonstrated a strong positive association between levels of free testosterone and miR-155, miR-27b, miR-103 and miR-21 [1]. Interestingly, Xiong et al. reported that the chance of PCOS development has been reduced by 0.01-fold for every elevated fold expression of miR-23a [50]. Lower oestrogen synthesis has been linked to the over-expression of miR-181a and miR-378 via the downregulation of aromatase

miRNA	Place of detection	Proposed function
miRNA-376	GC [4]	Modulate ovarian GC proliferation and/or apoptosis
miRNA-155	GC/FF [42]	Modulate androgens
miRNA-33b-5p	GC [4]	Promote IR
miRNA-483-5p	GC [1]	Modulate ovarian GC proliferation and/or apoptosis
miRNA-145 [1]	GC	Modulate ovarian GC proliferation and/or apoptosis
miRNA-126-5p	GC [1]	Modulate ovarian GC proliferation and/or apoptosis
miRNA-29a-5p [1]	GC	Modulate ovarian GC proliferation and/or apoptosis
miR-29a	GC [1, 42]	Modulate ovarian GC proliferation and/or apoptosis, androgens
miRNA-224	GC [42]	Modulate ovarian GC proliferation and/or apoptosis, androgens
miR-485-5P [18]	GC	Modulate ovarian GC proliferation and/or apoptosis
miRNA-15a	GC [4]	Modulate ovarian GC proliferation and/or apoptosis, androgens
miR-1275	GC [20]	Modulate ovarian GC proliferation and/or apoptosis, androgens
miR-23a	GC [4]	Modulate ovarian GC proliferation and/or apoptosis
miR-27a	GC [4]	Modulate ovarian GC proliferation and/or apoptosis
miR-182	GC [4]	Modulate ovarian GC proliferation and/or apoptosis, steroidogenesis
miR-200 [14]	GC	Modulate ovarian GC proliferation and/or apoptosis
miR-3940-5P [26, 27]	GC	Modulate ovarian GC proliferation and/or apoptosis
miR-486-5P [26, 27]	GC	Modulate ovarian GC proliferation and/or apoptosis
miR-206	GC [19, 26]	Modulate ovarian GC proliferation and/or apoptosis
miR-204	GC [19, 26]	Modulate ovarian GC proliferation and/or apoptosis
miRNA-320a [1]	Cumulus cells	Inhibit steroidogenesis
miRNA-509-3p	Cumulus cells [1]	Modulate androgens
miR-141 [14]	GC/TC	Modulate ovarian GC proliferation and/or apoptosis, steroidogenesis
miRNA-92b	GC/TC [1]	Modulate androgens
miRNA-222 [42]	TC/serum	Modulate androgens
miRNA-92a [1]	TC	Modulate androgens
miR-502-3p	TC [14]	Modulate androgens
miR-200a [14]	TC	Modulate androgens
miR-200c [14]	TC	Modulate androgens
miRNA-233	Adipose tissues [1]	IR
miRNA-93 [1]	Adipose tissues/GC	Modulate ovarian GC proliferation and/or apoptosis
miRNA-320	Adipocytes/FF/GC [1, 4, 42]	Modulate androgens
miRNA-132 [1, 42]	GC/ FF	Modulate androgens

miRNA	Place of detection	Proposed function
Let7-b [1, 11]	FF/GC	Modulate ovarian GC proliferation and/or apoptosis
miR-382-5p [37]	FF	Modulate androgens
miR-24 [42]	FF	Modulate androgens
miRNA-32 [38]	FF	Modulate androgens
miRNA-34c [38]	FF	Modulate androgens
miRNA-135a	FF/GC [38]	Modulate androgens
miRNA-18b [42]	FF/GC	Modulate androgens
miR-21 [42]	FF/GC	Modulate androgens
miRNA-30a [42]	FF	Modulate androgens
miRNA-140 [42]	FF	Related to ovarian follicle development
miR-9 [42]	FF/GC	Modulate androgens
miR-127-3p	FF [37]	IRs
miR-151 [42]	FF	Modulate androgens
miR-151-3p [3]	FF	Modulate androgens
miR-146a [42]	FF/serum	Modulate androgens
miR-518-3p [3]	FF	Modulate androgens
574-3p [3]	FF	Modulate androgens
miR-518	FF	Modulate androgens
miRNA-103	Blood/GC [42]	Modulate androgens
miRNA-27b [1]	Blood	Modulate androgens

Table 1.
Circulating and ovarian miRNAs that been proposed altered in PCOS.

enzyme [51, 52]. It is intriguing to note that miR-200b is involved in ovulation at the hypothalamo-pituitary ovarian axis level and is a downstream target for AR; such information may indicate that miR-200 plays a role in HA and PCOS [14].

HA is associated with an array of pathological changes that interact to promote the development of PCOS. Of these changes, hyperinsulinemia, IR and dyslipidaemia are well characterised.

2.2.2 miRNAs and insulin resistance (IR) and/or hyperinsulinemia in PCOS

IR plays a major role in the pathogenicity of PCOS with a pervasiveness of 70% among patients. It is known to be associated with impaired glucose metabolism, T2DM, metabolic syndrome, dyslipidaemia and increased risk of CVD. Hyperinsulinemia contributes to the increased ovarian steroidogenesis and androgen production via insulin growth factor-1 (IGF-1) receptor to initiate LH-induced TCs excess androgen secretion [53]. IR synergistically stimulated androgen via increasing CYP17 enzyme activity leading to lower SHBG levels and thus increase free testosterone levels [4, 54]. IGF-1 signalling, peroxidase proliferator receptor (PPAR) and angiopoietin, have been associated with PCOS and are potentially regulated by miR-223 [41]. miR-483-5p has been shown to lower IRs in women with PCOS [28]. It has been suggested that miR-320 and miR-132 can play a role in modulating insulin

resistance [43]. The author predicted that RABSB and HMGA2 are the target genes for miR-320 and miR-132, respectively, and that both gene expressions are altered by the reduced levels of miR-320 and miR-132 in the FF of PCOS individuals [43]. Increased expressions of miR-194, miR-193b and miR-122 have been noted in PCOS patients when compared with controls. The upregulation of these three miRNAs was found to target several pathways including insulin signalling pathway [22]. A strong association has been reported between the expression of miR-93 and miR-33b-5p and the development of IR in women with PCOS. They have been shown to exert their effect through the downregulation of glucose transporter 4 (GLUT4) expression [10, 55]. A significant positive correlation has been found between miR-222 expression and serum insulin [48]. Lin et al. [2] reported that 200a expression is associated with IR and T2DM. miR-1, miR-29, miR126 and miR-19a have been postulated to regulate glucose uptake via modulating PI3K [56].

All these findings indicate a cross talk between HA and hyperinsulinemia in PCOS. miRNAs involved in the regulation of these two dominant features are key for the development of specific markers for the diagnosis and treatment of the syndrome.

2.2.3 miRNA and dyslipidaemia in PCOS

The prevalence of hyperlipidaemia is 70% in PCOS. It is generally manifested by lipid profile dysfunctions, including increased levels of low-density lipoprotein cholesterol (LDL-C), reduced levels of high-density lipoproteins cholesterol (HDL-C) and high triglycerides [57]. Even though obesity is not indicative of PCOS, however, visceral adipose tissues (VATs) were found higher in patients with PCOS, whether they are obese or not, when compared with a control group [33]. To date, studies have found a close correlation between androgen excess, obesity and PCOS [5]. Androgen excess promotes the deposition of abdominal visceral fat, which in turn drives the secretion of adrenal and ovarian androgens via several pathways induced by adipose tissue dysfunction [58].

Current data on the interaction between miRNAs, dyslipidaemia and PCOS is sparse. In animal models, miR-33 has been shown to exert some control on LDL-C secretion and modulation of cholesterol biosynthesis [4, 59]. In addition, miR-128-1, miR-185 and miR-148a were reported to significantly decrease LDL-C levels, whereas miR-148a expression increased HDL-C [60]. The target genes for these miRNAs were adenosine triphosphate (ATP) binding cascade transporter A1 (ABCA1) and ABCG1 [60]. Furthermore, miR-34c-5p, miR-760, miR-597 and miR-1468 were found to be positively linked to hirsutism score. miR-597 and miR-1468 were shown to target the androgen receptor pathway [58]. Murri et al. [58] identified a group of miRNAs that are differentially expressed among PCOS patients. Of which, miR-30c-5p, miR-34c5p, miR-151a-5p, miR-193a-5p, miR-199a-3p, miR-1539, miR-26a-5p, miR-107, miR-142-3p, miR-126-5p and miR-598 were shown to correlate either positively or negatively with abdominal adiposity, obesity and metabolic dysfunction. Among all, miR-107, miR-30c-5p, miR-199a-3p and miR-26a-5p were noted to significantly associate with fatty acid biosynthesis and metabolism, and their expressions were found reduced in PCOS. The same group demonstrated further an increased expression of miR-338-3p, miR-365, miR-223-3p and miR-197-3 in obese PCOS patients when compared with the control subjects. All the reported miRNAs were correlated with androgen excess, glucose metabolic index and BMI [58]. Furthermore, miR-548-3p and miR-34c-5p expressions were also found to increase in PCOS individuals when compared with controls. Both miR-548-3p and miR-34c-5p markedly correlate with fatty acid biosynthesis and metabolisms [58].

Taken together, miRNAs play a pivotal role in the interaction of HA, hyperinsulinemia, dyslipidaemia and PCOS. Therefore, targeting miRNAs involved in the regulation of these features can shed light on the clinically relevant diagnostics and therapeutics markers of PCOS.

3. Considering miRNAs as a potential therapeutic target in PCOS

Based on the current knowledge presented in previous sections, miRNAs seem to be associated to the development of PCOS. However, no reports are available postulating the use of drugs to target miRNAs as a potential treatment option. Current guidance on disease management options is only focusing on improving prognosis and mitigating the symptoms. Of interest, PCOS patients are mostly undergoing in vitro fertilisation and thus involved in pharmacological protocols to support their fertility treatment. Available treatment options include the combined Letrozole and clomiphene citrate treatment, which is typically used for ovulation induction. Oral contraceptives (OCPs) control ovulation and prevent cyst formation. Anti-androgen drugs such as flutamide and spironolactone are used to manage increased androgens-related problems. Metformin is an insulin sensitivity agent that can improve the life quality for these patients by reducing serum insulin and androgen levels and elevating SHBG [4, 9, 61]. Polyphenols are a natural compound that has been used traditionally as a treatment option for several conditions; however, their limited bioavailability restricted their use. It has recently dragged some attention as a therapeutic target to ameliorate PCOS symptoms and reverse expression of crucial genes; nevertheless, more data are required before their administration into clinical use [61].

Studies focusing on therapeutics that may target small molecules such as miRNAs are rarely reported. Recently, metformin administration has been found to either positively or negatively target some miRNAs. Bao et al. demonstrated that metformin can inhibit markers for pancreatic cancer stem cells via the upregulation of miR-26a [62]. In addition, another study reported that metformin decreased the expression of miR-221 and miR-222 in T2DM patients [4, 9, 63]. Even more, metformin with sitagliptin combined therapy has been proposed as a treatment strategy to ameliorate PCOS in patients with IR [64]. This strategy was found to reduce GC apoptosis via mediating IncRNA H19 expression [33, 64].

Incretins-based therapies have been recently discovered to influence miRNAs expression and its potential for the management of PCOS. Glucagon-like peptide 1 agonist receptor agonist (GLP-1RA) decreased blood sugars via the downregulation of miR-375 and miR-23 and the increased expression of miR-192, miR-132 and miR-27a [65].

Treatment approaches targeting miRNAs associated with the metabolic feature of the disease may improve the clinical outcomes. miRNAs regulating androgen hormones are worthy at the top of the list. However, it is still a rich field of scientific research and validation.

4. Clinical application of miRNAs is still limited

Despite the hope, great effort and the scientific work done to clarify the role of miRNAs in PCOS, it is still a long way to go before it is possible to utilise miRNAs as a

diagnostic and therapeutic tool. It is perhaps due to the complexity of both miRNAs and PCOS.

The pathogenicity of PCOS involves several phenotypes described by the Rotterdam criteria and other less sever criterion, thus providing difficult and ununited diagnosis. In addition, the fact that one mRNA maybe regulated by multiple miRNAs and that miRNAs can modulate each other complicates the matter even further. Moreover, most of the studies performed are limited by the sample size and sometimes with contradicting results. Furthermore, metformin is mostly taken by PCOS individuals during their infertility treatment and has been shown to influence miRNA expression, thus may false mask facts and lead to contradicting results. Therefore, it is imperative to deepen the current research to develop consistent and reliable miRNA-based diagnostics and therapeutics.

5. Conclusion and future direction

Taken together, miRNAs play a key role to fine-tune events leading to ovarian cell apoptosis, proliferation, follicular development, HA, altered insulin and dyslipidaemia in PCOS. Yet, it is not possible to determine whether miRNAs-altered expression is a cause or is an aftereffect event of the syndrome. Furthermore, the dynamic expression and action of miRNAs complicate facts further. Thus, further functional studies on mRNA-miRNA and the interplay between epigenetic regulation and altered miRNA expression are required to highlight the pathogenicity of the disease. It is, however, possible to use miRNAs as biomarker to categorise and identify PCOS sub-phenotypes. miRNAs are a promising candidate to modulate pathways involved in the pathogenicity of this syndrome.

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Chapter 8

Role of Oxidative Stress and Carnitine in PCOS Patients

Bassim Alsadi

Abstract

Polycystic ovary syndrome (PCOS) is a common female endocrine and reproductive system disorder which is found in 6–10% of the female population. PCOS is considered a multifactorial metabolic disease characterized by several clinical manifestations, such as hyperandrogenism, polycystic ovaries and ovulatory dysfunctions. PCOS patients have an increase in the oxidative stress with generation of excessive amounts of reactive oxygen species (ROS) and reduction of antioxidant capacity. Oxidative stress is defined as the imbalance between the production of free radicals and the ability of the organism to defend itself from their harmful effects damaging the plasma membrane, DNA and other cell organelles, inducing apoptosis. Oxidative stress markers are circulating significantly higher in PCOS patients than in healthy women, so these can be considered as potential inducers of the PCOS pathology. Therefore, the central role of the oxidative stress may be involved in the pathophysiology of various clinical disorders including the PCOS. This chapter reviewed the role of oxidative stress and carnitine in PCOS patients, indicating the beneficial action of the carnitine pool, and L-carnitine contributes to restore the energy balance to the oocyte during folliculogenesis and maturation, which represent an important strategy to improve the intraovarian environment and increase the probability of pregnancy.

Keywords: polycystic ovary syndrome, ultrasound, anovolution, infertility, hyperandrogenism, oxidative stress, carnitine pool, insulin resistance, advanced glycation end products, RAGE (receptor for AGEs), hyperinsulinemia, reactive oxygen species (ROS)

1. Introduction

Polycystic ovary syndrome (PCOS) is a common female endocrine and reproductive system disorder which is found in 6–10% of the female population [1].

In general, it is considered a multifactorial metabolic disease characterized by several clinical manifestations such as hyperandrogenism, polycystic ovaries aspects on ultrasound and ovulatory dysfunctions which makes it the most common cause of anovulation infertility in women, but also from metabolic problems such as obesity, insulin resistance, hyperinsulinemia and type II diabetes which may enhance cardiovascular complications and other neurological and psychological implication such as anxiety and depression [2, 3].

The carnitines are essential in the metabolism of fatty acids and can act to protect from mitochondrial damage and altered energy balance conditions such as those present in polycystic ovary syndrome (PCOS) as also highlighted by the reduced levels of L-carnitine in the serum of patients with this disease.

Restoring the energy balance and adequate energy reserves to the oocyte during folliculogenesis and maturation can represent an important strategy to improve the intraovarian environment and increase the probability of pregnancy. In this context, metabolic compounds, such as carnitines, with positive effects on mitochondrial activity and free radical scavenging, can contribute to mitigate the effects of PCOS.

2. Clinical remarks of PCOS

In the last decade there has been a plenty of discussion regarding the pathogenesis of PCOS, the causes are not yet known, but environmental and genetic factors may be involved [4, 5].

In particular, the genetic abnormalities appear to play a key role in the metabolic complications with a high rate of hyperandrogenism and type II diabetes in first degree relatives of women with PCOS [6, 7].

Recently, some studies have indicated that a defect in insulin action could be the primary cause of PCOS [8, 9].

Other studies have instead observed how important the role of socio-economic status and unhealthy life style, which includes smoking, poor diet, poor exercise and obesity [10, 11]. Furthermore, other studies have suggested that ethnic background may also be associated PCOS probably due to the increased number of insulin resistance and type II diabetes in this population [12, 13]. Polycystic ovary syndrome is the most common cause of menstrual irregularity leads to infertility and it is estimated that 90% of anovolution cases are caused by PCOS [14].

In addition to endocrine and reproductive clinical findings, PCOS also leads to consequences on mental health. Studies showing the correlation between PCOS and reduced quality of life [15, 16] with the increase in anxiety and depression [17]. This is not surprising, since the main phenotypes of this syndrome (obesity, infertility and hirsutism) are major problems that can cause psychological stress. Neuroendocrine dysfunction in gamma-aminobutyric acid (GABA) signaling and neuronal androgen receptors that might alter hypothalamic sensitivity and lead to an impairment of estradiol and progesterone feedback. Elevated concentrations of GABA in the cerebrospinal fluid of women with PCOS, GABA seems to exert an excitatory effect on GnRH neurons and this leads to greater secretion of LH by the pituitary gland, as occurs in PCOS [18, 19].

The metabolic implications of PCOS increase the risk of cardiovascular complications in PCOS patients [20]. Chronic anovulation in PCOS patient may lead to endometrial hyperplasia increasing the risk of endometrial cancer. Obesity, insulin resistance and type 2 diabetes associated to PCOS will enhance the risk of endometrial cancer in PCOS patients [21, 22].

PCOS patients have an increased risk of type II mellitus [23], in addition, insulin resistance plays a central role in the pathogenesis of PCOS [24] as it provokes hyper-insulinemia and accelerates the over-production of androgens in the ovary. Hyper-insulinemia which, in turn, contributes to the development of diabetes and dyslipidemia [25].

3. Oxidative stress in PCOS

Central role of the oxidative stress may be involved in the pathophysiology of various clinical disorders including the PCOS.

PCOS patients have an increase in the oxidative stress with generation of excessive amounts of reactive oxygen species (ROS) and reduction of antioxidant capacity [26].

Oxidative stress is defined as the imbalance between the production of free radicals and the ability of the organism to defend itself from their harmful effects damaging the plasma membrane, DNA and other cell organelles, inducing apoptosis [27]

Oxidative stress markers are circulating significantly higher in PCOS patients than in healthy women, so these can be considered as potential inducers of the PCOS pathology [28].

4. Advanced glycation end products (AGEs)

Among the most important post-translational modifications is the non-enzymatic modification of proteins, lipids and nucleic acids with glucose and their consequent conversion into AGEs.

AGEs (advanced glycation end products) therefore represent the final products of a chemical process known as the Maillard reaction, in which carbonyls of glucose or other reactive sugars react non-enzymatically with amino groups of proteins. The further reorganization of which leads to the formation of the Amadori product: the proteins containing this product are known as glycated proteins and the process of formation is known as glycation. Depending on the nature of these glycation products, protein adducts or protein cross-linking are formed, giving rise to the AGEs [29].

The end product of this reaction (AGE), in turn, induce oxidative stress and accelerate the Maillard reactions ultimately leading to inflammation and the propagation of tissue damage [30–32].

Advanced glycation end-products (AGEs) such as glycated hemoglobin commonly used in clinical practice as a marker of hyperglycemia is an Amadori product implicated in the development diabetes mellitus [32].

AGEs can be taken exogenously, through the consumption of food and smoke, or produced endogenously. In fact, in physiological conditions, AGEs are formed very slowly while, in particular conditions like hyperglycemia, insulin resistance, obesity, aging, oxidative stress and hypoxia, their formation process is accelerated [33].

Any accumulation of AGE is associated with various diseases, such as diabetes mellitus type 2, metabolic syndrome, cardiovascular diseases, ovarian aging, neurodegenerative disorders, obesity and PCOS [33–35].

Once formed, AGEs can damage cellular structures through a number of mechanisms, including the formation of cross-links between key molecules of the basement membrane of the extracellular matrix and interaction with receptors on cell surfaces, leading to this way to alteration of cellular function [36, 37].

However, the AGE content in the body is not defined only by their rate of formation, but also from rate of removal. The body cells in fact have developed pathways of detoxification against the accumulation of AGE [38].

The interaction between circulating AGEs and RAGE (receptor for AGEs) will trigger and enhance the pro-inflammatory state, cell toxicity cell and damage **Figure 1** [40].

RAGE is a transmembrane receptor and is expressed in numerous tissues including ovaries, heart, lung and skeletal muscle, but also in monocytes, macrophages and lymphocytes [41]. In physiological conditions this receptor is down-regulated while with aging its expression increases, probably due to the accumulation of ligands which, through positive feedback, regulate the expression of receptor itself [40–42].

In conditions like diabetes, inflammation, atherosclerosis and PCOS, there is a marked induction of RAGE due to the action of ligands and the numerous mediators activated by inflammatory cells **Figure 2** [44].

5. Factors that induce the production of advanced glycation end products (AGEs)

AGE levels in blood and tissues depend on endogenous sources (chemical reactions) and exogenous sources (diet and smoking). In particular foods rich in protein and fat, like meat, cheese and egg yolk, they are in fact rich in AGE, moreover, cooking methods (such as high temperatures) also increase their concentration drastically [45].

Smoke is another exogenous source of AGE and it has in fact been seen that the serum levels of AGE in smokers are significantly higher compared to non-smokers [46].

The presence of AGE in ovarian tissue, together with an altered metabolic profile and elevated testosterone levels therefore provides evidence for a double effect of the AGE taken with the diet on reproductive and metabolic function [39].

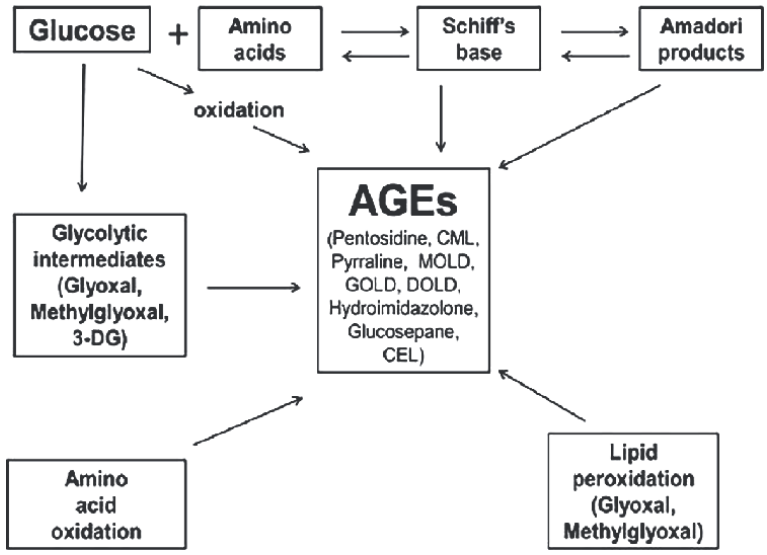

Figure 1.
Advanced glycation end products and their relevance in female reproduction [39].

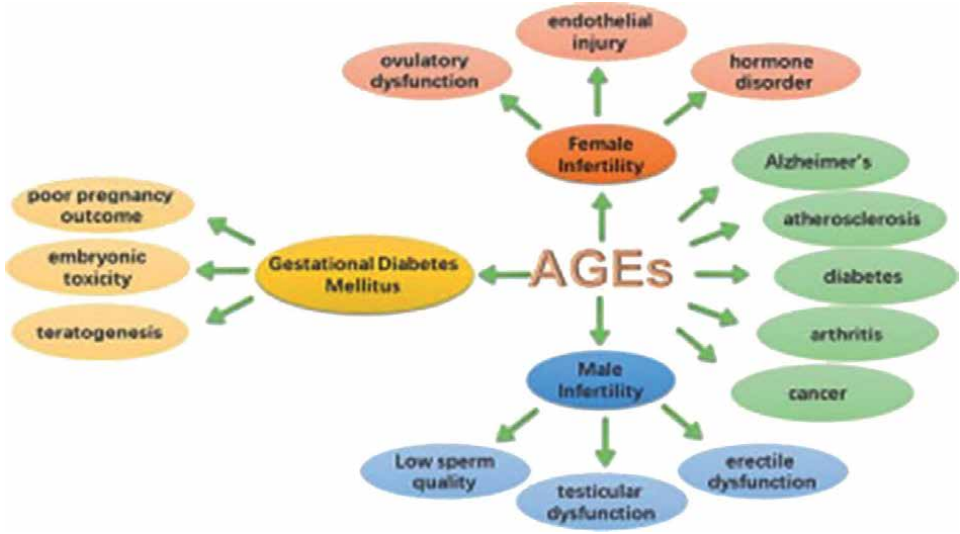

Figure 2.
The role of advanced glycation end products in human infertility [43].

6. Correlation between AGEs and feature of PCOS

PCOS has been defined as a disorder due to an excess of androgens and insulin resistance, about 50–70% of women with PCOS have a certain degree of resistance to insulin, which is defined as a state in which more insulin is required than the normal to obtain an appropriate response [47]. Besides contributing to PCOS-associated hyperandrogenism, insulin resistance is also linked the development of impaired glucose tolerance and type 2 diabetes mellitus [48], in both obese and non-obese women with PCOS [49]. It is unclear whether hyperandrogenism is the result of hyperinsulinemia or vice versa [50]. Both insulin-like growth factor 1 (IGF-1) and insulin are potent stimulators of production of ovarian androgens, an action probably mediated by the insulin receptor [50, 51], furthermore, it is possible that the increase of circulating insulin levels potentiate the effect of luteinizing hormone (LH) on cells of the ovarian theca. Another mechanism of possible hyperandrogenism observed in the PCOS is the insulin-mediated inhibition of sex hormone binding globulin, which results in an increase of free androgens [52].

Since oxidative stress and inflammation are closely associated with insulin resistance, it is conceivable that the AGE-RAGE system may play a role in pathogenesis of insulin resistance observed in PCOS [30], regardless of circulating glucose levels, weight and obesity. A study conducted by Cai et al. has in fact identified the AGEs as a risk factor for insulin resistance independent of over-nutrition in non-obese mice [53], with such insulin resistance that occurred before changes in blood glucose levels. Additionally, recent work on overweight women reported that a low AGE diet improves insulin sensitivity [54].

About 30–75% of women with PCOS are obese [55] and such patients are likely at more risk to suffer from severe consequences than PCOS, such as hyperandrogenism and metabolic syndrome, compared to patients with a normal BMI [1, 56]. Moreover,

it has been shown that modest weight loss regulates menstruation, improves reproductive performance and hirsutism, reduces serum androgen and insulin levels and improves the index of insulin sensitivity in women with PCOS [1].

In addition, the distribution and morphology of adipose tissue appear to contribute significant to the pathophysiology underlying PCOS: most affected women in fact, it presents an abdominal distribution of adipose tissue (central obesity) independent of BMI, which is an effect probably associated with the high amount of circulating androgens [56].

Circulating AGEs correlate with indicators inflammation, such as C reactive protein (CRP), and with oxidative stress [57]. In addition, the accumulation of AGE in the tissues induces cellular oxidative stress and promotes inflammation, thus increasing the vulnerability of the target tissues [58]. The dietary restriction of AGE, in fact, is associated with a significant reduction in inflammatory markers, such as plasma CRP, TNF-α (tumor necrosis factor-α) and VCAM-1 (vascular cell adhesion molecule-1) [59]. Furthermore, AGEs are directly correlated with the physiology of adipocytes as AGEs may also stimulate adipogenesis [60].

Patients with classic PCOS phenotype show alterations in the follicular fluid intermediate metabolites and the cumulus cells have an increase in oxidative stress, which causes the alteration of processes of follicular growth and oocyte development, causing the reduction in the pregnancy rate [61].

7. AGE-RAGE system in serum and ovarian PCOS

Insulin resistant women with PCOS without hyperglycemia have elevated levels serum of AGE and the expression of RAGE in circulating monocytes [44]. Furthermore, serum AGE levels are positively correlated with levels of testosterone, free androgens and insulin [50]. An increase in the serum AGE levels suggesting that serum AGEs are high in PCOS regardless of the presence of insulin resistance [62]. Recent studies have also shown that RAGE and AGE-modified proteins are expressed in human ovarian tissue [35, 63]. Specifically, women with PCOS have an increase in the expression of AGE and RAGE in the theca and granulosa cell layers, compared to healthy women [34, 64].

The AGE-RAGE system may be responsible for the failure of ovulation characteristic of PCOS: in a model of human cell lines of granulosa, observed that the AGE interfere in vitro with the action of LH leading to altered follicular development and therefore the dysfunction ovulatory associated with PCOS [65]. The AGEs within the ovary alter glucose metabolism and the folliculogenesis, the AGE could be responsible for the reduction of glucose uptake by granulosa cells, with consequent alteration of follicular growth [66].

The relationship between the AGE-RAGE system and infertility was also documented: AGEs have a negative effects on the reproductive outcome in women undergoing ART (assisted reproduction technology), AGE high levels in NON PCOS women appear to be related to the decrease in ovarian reserve and abnormal folliculogenesis. The pathological significance of these inflammatory AGE molecules, which are harmful to the follicles, clearly requires further investigation, but the identification of specific AGEs could offer potential therapeutic options for treating the decreased response ovarian **Figure 3**.

Intra ovarian dyslipidemia is probably a consequence of the changes associated with the metabolism in the follicles [67]. In addition, the exposure of cumulus-oocyte complex to a high lipid concentrations are known to have negative influences on oocyte maturation [68].

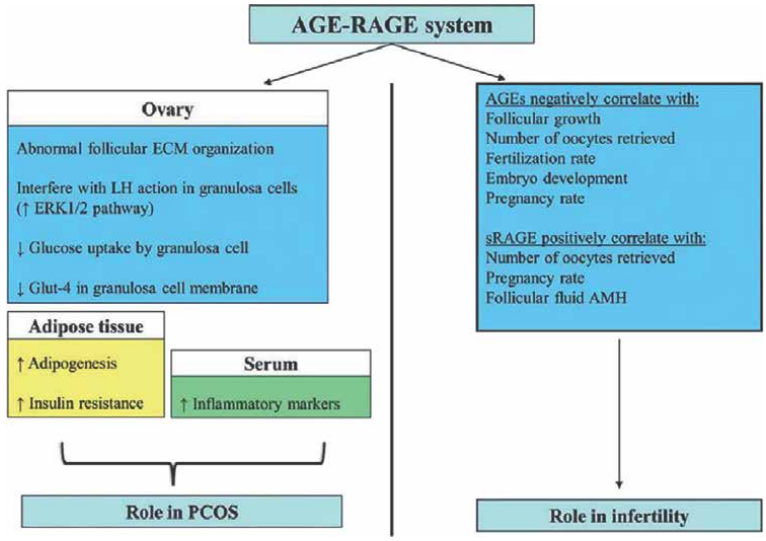

Figure 3.
Relationship of the AGE-RAGE system with PCOS and infertility [39].

8. Role of Carnitine in PCOS and female infertility

Levocarnitine (L-carnitine) plays a central role in the cellular energy metabolism as it is an essential molecule for the transport of long-chain fatty acids across the internal mitochondrial membrane. It was first isolated in 1905 in bovine muscle [69] and only the L isomer is bioactive. The carnitines as a whole they belong to a special class of nutrients called "quasi-vitamins" or "Conditionally essential" nutrients [70]. L-carnitine can be synthesized endogenously or taken with the diet, in particularly through meat and dairy products [71], hence its homeostasis reflects the balance between endogenous biosynthesis, absorption from the diet and renal reabsorption [72].

Numerous clinical studies have reported that the administration of L-carnitine (LC) and/or acetyl-L-carnitine (ALC) alleviates some effects of PCOS resulting in an increase reproductive outcome [73–77].

Both LC and ALC are commonly used in reproductive biology to improve mitochondrial function in the treatment of female infertility [78, 79]. Specifically, ALC is predominantly used for its antioxidant and anti-aging effect, while the use of LC to promote capacity of the body to oxidize fat cells to produce energy and burning fat [80]. LC also prevents DNA fragmentation induced by the harmful actions of free radicals [81].

Numerous studies have indicated that administration of L-carnitine (LC) and its acetylated form, acetyl L-carnitine (ALC) improves conditions such as PCOS [73], endometriosis [82] and amenorrhea [83]. In addition, carnitines increase levels of gonadotropins and sex hormones, as well as improve oocyte health **Figure 4** [83].

The administration of ALC instead increases the serum levels of other reproductive hormones such as estradiol, progesterone and luteinizing hormone (LH) and decreases prolactin [83, 85]. Hence, through their indirect endocrine effect, carnitines can prevent PCOS, amenorrhea and other pathological conditions related to the reproductive cycle female.

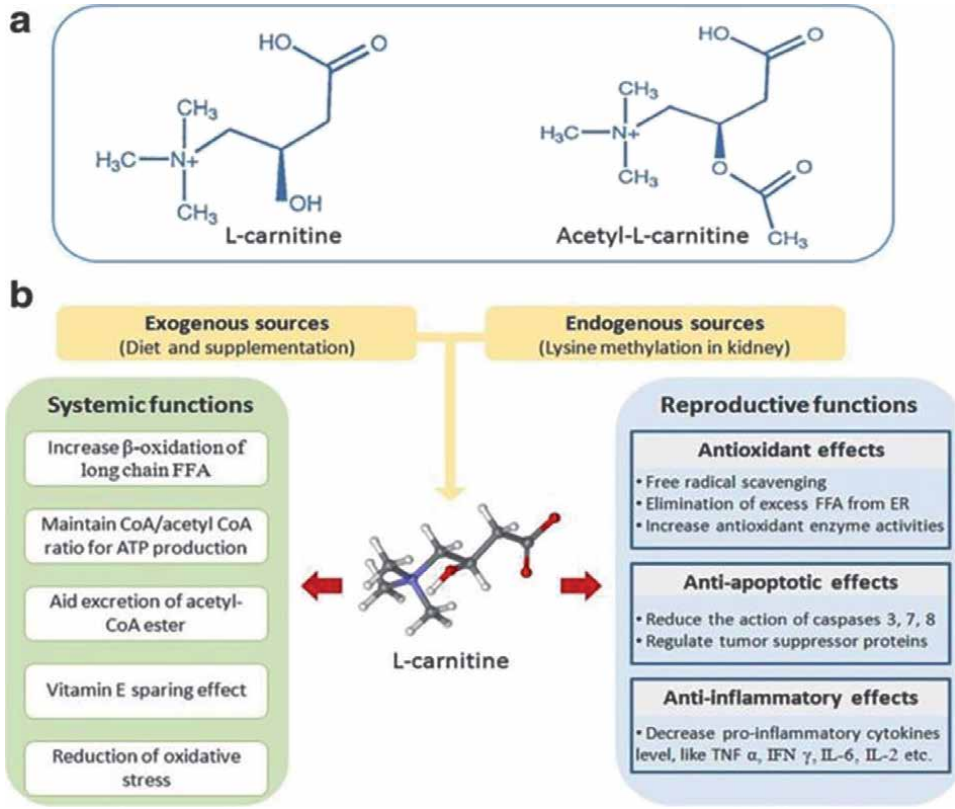

Figure 4.
(a) Molecular structures of L-carnitine and acetyl-L-carnitine, (b) systemic and reproductive functions of L-carnitine. CoA, coenzyme A; ER, endoplasmic reticulum; FFA, free fatty acid; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor [84].

LC and ALC also affect the hypothalamic-pituitary-gonadal (HPG) axis, inducing secretion of reproductive hormones [83, 85, 86]. Among the neural centers, the concentration of LC is higher in the hypothalamus [87]. LC reduces the death rate of nerve cells and the damage associated with aging [88], thanks to its cholinomimetic activity [89]. It also increases the secretion of gonadotropin-releasing hormone (GnRH) from part of the hypothalamus, inducing the depolarization of hypothalamic nerve cells to increase its secretory activity **Figure 5** [90, 91].

Regarding PCOS, Samimi et al. observed that supplementation with LC (250 mg oral L-carnitine supplementation for 12 weeks) leads to a significant reduction in body weight, body mass index and waist and hip circumference decreasing blood glucose levels and favors the contrast of insulin resistance [73], which may be attributed to the increase in β-oxidation of fatty acids and the metabolic rate base line induced by LC [74].

As women with PCOS present also an imbalance between male and female hormones as their ovaries tend to produce androgens in excessive quantities, such phenomena of hyperandrogenism and/or insulin resistance in non-obese women with PCOS may be associated with the lowering of serum levels of LC [75]. Recent studies based on mass spectrometry confirmed altered fatty acid levels and carnitine in the serum of PCOS patients [92].

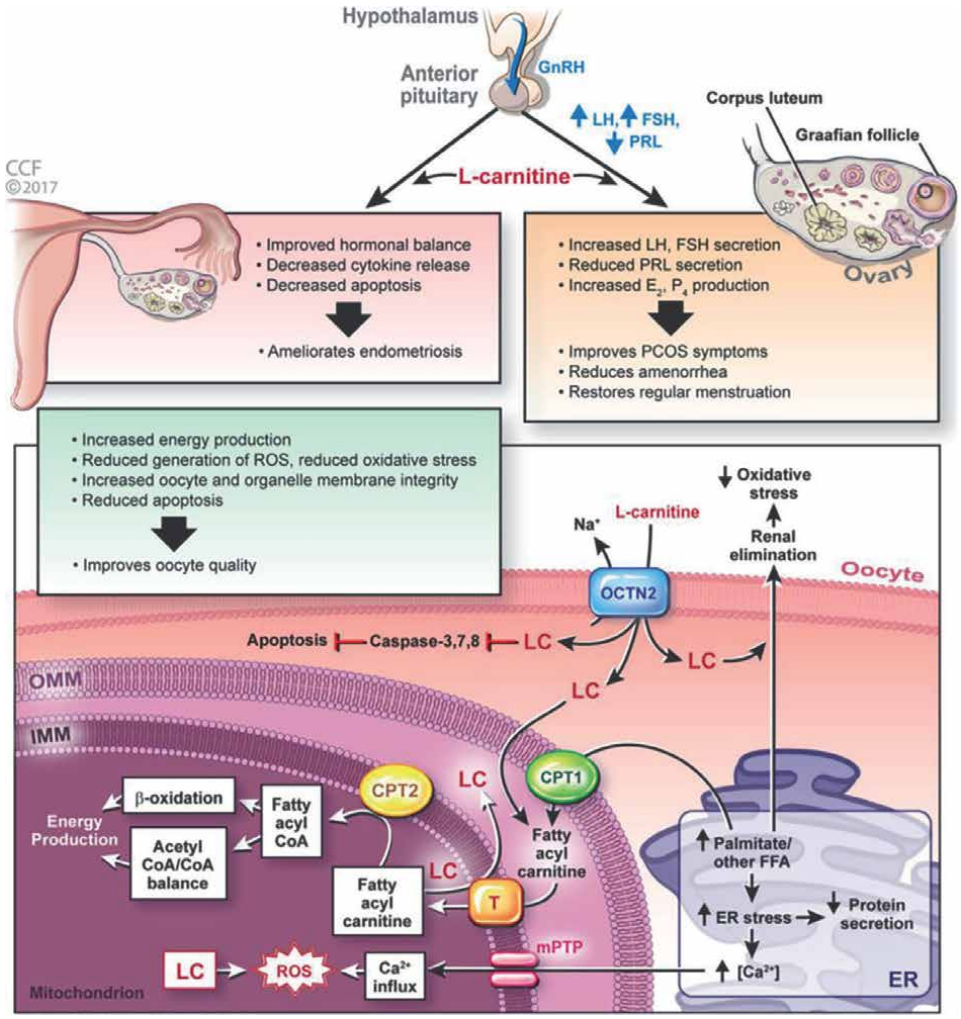

Figure 5.
Mechanism of L-carnitine action on female fertility [84].

9. Carnitine and human assisted reproduction

Due to their beneficial effects on female fertility, carnitines have been used in numerous in vitro studies focused on improving the health and maturation of oocytes, embryonic development in assisted reproduction, in fact they allow to reduce the delay in embryonic development due to ROS, the fragmentation of the DNA and the development of an abnormal blastocyst due to prolonged culture [93, 94].

It has been observed that during oocyte development the cumulus-oocyte complex (COC) plays an essential role in lipid metabolism and therefore in energy production: therefore, in the oocyte, the maintenance of a correct lipid metabolism without or with the minimum generation of free radicals is necessary to preserve its quality [95].

LC is essential for maintaining cellular energy balance and to reduce oxidative stress [96] and to minimize cell death by apoptosis [97], which is necessary for adequate growth of the oocyte and for the maturation of the blastocyst. LC promotes

the lipid metabolism of the cumulus-oocyte complex (COC), which is one of the main regulators of oocyte maturation, by transferring the fatty acids in the mitochondria and facilitating their β-oxidation [95].

Carnitine also has an anti-inflammatory effect as the integration of diet with LC decreases the anti-proliferative effect induced by the presence of interleukins such as TNF-α and its detrimental action reducing the consumption of glucose of the embryo in its early development [98] and reducing growth of the inner cell mass and trophoectoderm in the blastocyst, which leads to delayed embryonic development and reduction of vitality of the embryo [99, 100].

It has been observed that the integration of the culture medium with ALC stabilized the mitochondrial membrane, increased the energy supply to the organelles and protected the developing embryo preventing its fragmentation [101]. Furthermore, the integration of the culture medium with LC in addition to showing anti-apoptotic effects, increases the rate of development of blastocysts [97].

Supplementation of the culture medium with LC during the in vitro maturation of the oocytes favored the acquisition of competence for development, as it improved cytoplasmic and nuclear maturation and reduced ROS levels in the culture medium, showing an antioxidant effect [102].

Furthermore, women with endometriosis have a marked increase in TNF-α concentration in the granulosa cells [103–106], which leads to the reduction in the size of the inner cell mass and in the proliferation of the trophoectoderm in the blastocyst, it was observed that the integration of the culture medium with LC allowed to neutralize the antiproliferative effect of TNF-α and to limit DNA damage during embryo development [93]. LC also had a protective effect against oocytes and embryos against the toxic effects of peritoneal fluid in women with endometriosis, reducing apoptosis levels in embryos and enhancing the microtubular structure [107].

Another typical feature of PCOS is chronic anovulation and the standard approach for the treatment of women with anovulation infertility consists of administration of clomiphene citrate to induce ovulation, however, some women fail to ovulate despite taking increasing doses of clomiphene citrate and, therefore, defined as clomiphene citrate-resistant PCOS. The administration of clomiphene citrate together with LC increases both the ovulation and pregnancy rate in women with clomiphene citrate-resistant PCOS. In addition, the integration with L-carnitine increases the number of follicles capable of ovulating (diameter ≥17 mm), and oocyte maturation, as well as serum levels of estradiol and progesterone [76].

An alternative treatment to induce ovulation in patients with citrate-resistant clomiphene PCOS consists of therapeutic treatment with gonadotropins; however, some of these women do not respond to both treatments, the addition of LC to therapy stimulates the growth of dominant follicles, favoring the pregnancy rate, it also increases the average thickness of the endometrium and the size of ovarian follicles [77].

10. Conclusion

The beneficial effects of carnitines on the reproductive system and ovarian function as well the differential action of the carnitine pool. The carnitines are essential in the metabolism of fatty acids and can act to protect from mitochondrial damage and altered energy balance conditions such as those present in polycystic ovary syndrome (PCOS). The L-carnitine contributes to restore the energy balance and provide

adequate energy reserves to the oocyte during folliculogenesis and maturation and can represent an important strategy to improve the intraovarian environment and increase the probability of pregnancy. In this context carnitines, with positive effects on mitochondrial activity and free radical scavenging, can contribute to mitigate the effects of PCOS.

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Chapter 9

Polycystic Ovary Syndrome Phenotypes and Infertility Treatment

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Abstract

The polycystic ovary syndrome (PCOS) includes different clinical, endocrine, metabolic, and morphological criteria in women of reproductive age and consequently different health risks in later life of a woman. Controversy and debates related to diagnostic criteria are constant and current worldwide. As a result of many proposals for PCOS diagnostic criteria, clinicians recognize four phenotypes of PCOS. PCOS is a frequent cause of infertility with an overall prevalence of 5–15% and counts for approximately 70% of all cases of ovulation disorders. There are many aspects of studying differences between PCO phenotypes and problems in infertility treatments. Ovulation induction is often used to treat anovulatory patients with PCOS, but many of these women fail to conceive and the next step in the treatment is assisted reproduction. The contribution of oocyte health to reproductive potential varies and largely depends on the PCOS phenotype and comorbidities associated with PCOS. Contrary to the previous one, PCOS phenotype is not significantly associated with the morphological quality of oocytes. It seems that a combination of hyperandrogenism and chronic anovulation is associated with a negative impact on the cumulative pregnancy rate in medically assisted reproduction.

Keywords: PCOS, PCOS phenotype, ART, ovulatory failure, reproductive hormone, *in vitro* maturation

1. Introduction

Management of PCOS (polycystic ovary syndrome) related to infertility, includes lifestyle changes, ovulation induction by pharmaceuticals, or assisted reproductive technology (ART) as an *in vitro* fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI) and *in vitro* maturation (IVM) of the oocyte. It can be followed by a "freeze-all" procedure. PCOS patients have a higher risk of developing ovarian hyperstimulation syndrome (OHSS), a life-threatening condition, therefore ART is no favored procedure in current international guidelines.

Hyperandrogenism, anovulation, and ovarian morphology are the basic determinants in the diagnosis of the polycystic ovarian syndrome (PCOS) according to international guidelines. Given the different clinical presentations in patients, the

criteria for the diagnosis of this condition are still discussed, as well as whether the syndrome involves several different diseases with the same clinical picture, as well as discussions about what is really a clinical picture of the polycystic ovary. Therefore, different approaches in the diagnosis and treatment of patients, have been proposed for different phenotypes of PCOS. The criteria for pre-recognition of this condition have been adopted for years by various authoritative bodies at international meetings, such as the National Institute for Health (NIH), Rotterdam consensus, Androgen Excess, and PCO Society, but there has been a constant difference over the mandatory criteria for PCOS [1]. An important starting point in the diagnosis was to exclude diseases of other endocrine glands (pituitary gland, thyroid, and adrenal gland), which give a similar clinical picture and can be confused with PCOS.

Ovulation disorder in the general population of women is estimated at 15% (12–18%) [2]. Regular menstrual cycles are not the exclusive evidence of ovulation, since in some women there is a "subclinical disorder" of ovulation that is proven only by serum values of progesterone in the middle lutein phase of the cycle (21–24.d.c. which must be >5 ng/mL). In the case of PCOS, almost 80% of patients have ovulation disorder [3].

Hyperandrogenism (hyperandrogenemia) implies clinical and/or biochemical evidence of elevated serum androgens, but the incidence in the general population of women is unknown. Hirsutism, androgenic alopecia, and acne are clinical manifestations of hyperandrogenism. The intensity of hirsutism differs ethnically and geographically, and it is desirable to develop population-specific criteria for hirsutism. Almost 70% of women with hirsutism have PCOS, 40% have severely expressed acne, and only 22% have androgenic alopecia [4]. Hyperandogenemia (biochemical hyperandrogenism) is determined by free testosterone and free androgen index (FAI—free androgen index) [5]. A total of 78% of patients with PCOS have hyperandrogenism and 40% in an unselected population of patients with BMI >25 [6].

Polycystic ovary morphology (PCOM) is evaluated by ultrasound examination based on the number of antral follicles (> of 20 per ovary) and/or on the basis of total ovarian volume (> 10 mL), where the frequency of the ultrasonic probe is an extremely important parameter. Based on these international criteria, the prevalence of PCOM in the population is 12.5% [7, 8]. Ultrasonic examination of nonselective population, based only on PCOM, significantly increase the incidence of PCOS and vice versa.

Thus, on the basis of the described criteria, four PCOS phenotypes with different prevalence in the general and separate population are defined, which are as follows [5]:

- Phenotype A (hyperandrogenism, anovulation, PCOM).
- Phenotype B (hyperandrogenism, anovulation).
- Phenotype C (hyperandrogenism, PCOM), ovulatory PCOS.
- Phenotype D (anovulation, PCOM), non-hyperandrogenemic PCOS.

Compared to phenotype C and D, patients with phenotype A and B (classical phenotype) are more often obese, with hirsutism, more likely to have insulin resistance, dyslipidemia, fatty liver, and metabolic syndrome in later life. The frequency of individual phenotype differs significantly in different populations with symptoms

of PCOS and also in the general population [9]. Each of the PCOS phenotypes has its own specifics in the treatment of impaired fertility.

2. PCOS phenotype and complications of treatment with medically assisted reproduction procedures

The first line of treatment of patients with PCOS is the induction of ovulation with clomiphene citrate or letrozole. *In vitro* fertilization (IVF) procedures are indicated when this initial treatment fails or in cases where the patient's partner has severe male infertility. Patients with PCOS phenotype A have significantly more frequent resistance to clomiphene despite increasing the dose of the drug through three consecutive stimulation cycles, compared to phenotype D (non-androgenic phenotype) [10].

Gonadotropin stimulation in patients with PCOS is associated with the development of a significantly higher number of follicles in the ovaries, as well as oocytes, a significantly higher number of developed embryos and embryos in excess for cryopreservation. Ovarian stimulation in these patients lasts longer and higher doses of gonadotropin are often required, which is associated with disorders of folliculogenesis caused by hyperandrogenism. Estimating the right dose of gonadotropin is the biggest challenge in the phase of ovarian stimulation and is often insufficient. The follicles do not grow, due to hyperandrogenism, and by increasing the dose, the ovary enters in hyperstimulation, which is an extreme of the ovarian response. A newer approach to ovarian stimulation with follitropin delta, based on the patient's body mass and AMH value, proved to be the best, especially in the PCOS patient population and has a significant reduction in the risk of ovary hyperstimulation. Patients with hyperandrogenism and polycystic ovarian morphology (phenotype A and C) have the highest risk of ovary hyperstimulation [11].

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication of ovarian stimulation, and PCOS patients have the highest risk for complications during the IVF (*in vitro* fertilization) procedure. The frequency of OHSS is from 3 to 6% of IVF cycles. Patients with antral follicles count >24, AMH concentration > 3.5 ng/mL, or estradiol concentration > 3500 pg., have a risk of developing OHSS. Clinical OHSS is recognized in three stages, and depending on the severity of symptoms, we distinguish between mild, medium severe, and severe types of hyperstimulation. Severe ovarian hyperstimulation can be a life-threatening condition, requiring hospitalization and treatment to maintain vital circulatory and pulmonary functions, and can also end with the death of a patient. Identification of patients at risk for OHSS is the basis of the strategy for the prevention of this serious iatrogenic condition and the safety of IVF procedures.

The protocol of choice for ovarian stimulation in patients with PCOS and risk for OHSS is an antagonistic protocol that can be fixed or flexible. In this stimulation, it is possible to achieve the final maturation of oocyte with GnRH (gonadotropin-releasing hormone) agonists, thereby avoiding the administration of hCG (human chorionic gonadotropin) injection, which is the basic molecule in the mechanism of development of OHSS in at-risk patients. In this way, the basic mechanism of vascular permeability and compromising circulation by leaking plasma from the vascular system into extracellular spaces are avoided. Those are signs of a more severe form of OHSS. Likewise, the stimulation cycle is abruptly "extinguished." Menstrual bleeding occurs within a few days after the application of the GnRH agonist. Harvested oocytes are fertilized by IVF/ICSI procedure and developed embryos are cryopreserved, most

often in the blastocyst stage, which represents the so-called "freeze-all" strategy that gives safety to the treatment of patients with PCOS. Embryo transfer is planned in the next cycle in which signs of hyperstimulation do not exist. Hormonal preparation of the endometrium, and ovarian stimulation, in this case, is not required.

Additional treatment of PCOS patients involves the use of various medications that have metabolic effects and that could significantly improve the treatment of these patients in IVF procedures by individualizing therapy. The fact is that within the PCOS population with the same PCOS phenotype, an individual woman may have a significantly different response to different types of treatments with respect to the unique hormonal/metabolic status associated with the PCOS phenotype as well. There is a large gap in the literature that indicates the need for new research and the need for an individual approach in the treatment of infertility of these patients.

Spontaneous abortions in patients with PCOS are more common compared to the general population and they are associated with insulin resistance, hyperandrogenism, and obesity. These conditions are very often associated with PCOS, but they are also separate risks for the spontaneous loss of pregnancy. Studies link spontaneous abortion to impaired endometrial receptivity and to more frequent embryo aneuploidy of patients with PCOS. In the Asian population of women with PCOS phenotypes who have hyperandrogenism (A, B, C types), a higher risk for spontaneous miscarriage after IVF procedures was observed than in phenotype D [12]. Impaired glucose and insulin metabolism at the endometrial level and excessive expression of androgen receptors in the endometrium are associated with a signal transduction disorder during the implantation process in patients with PCOS [13]. The causes of more frequent embryo aneuploidy in PCOS patients have not yet been clarified. There are assumptions that impaired glucose metabolism and steroidogenesis lead to DNA molecule instability [14].

3. PCOS phenotypes and the outcome of medically assisted reproduction procedures

During the stimulated IVF cycle, various indicators of quality and success of treatment are monitored. Among other things, these are the total dose of gonadotropin used for stimulation, the number of aspirated oocytes, the number of oocytes in metaphase II, the percentage of fertilization, the number of developed embryos on the 3rd day, the number of developed blastocysts on the 5th day, the number of cryopreserved embryos, the proportion of conceived pregnancies, the number of born children, etc. Since PCOS phenotypes imply hormonal and metabolic differences, the question arises whether the indicators of the course of treatment are different in patients with different PCOS phenotypes.

The results of the studies so far indicate significant differences in treatment between PCOS patients and women who do not have this syndrome and who in studies represent the usual control group. Studies most often follow PCOS patients as a single group. Different criteria for defining PCOS phenotype are associated with problems of analysis and comparison of parameters that monitor the course and outcome of the IVF procedures in different studies [15]. There are two fundamental factors that are most often analyzed and compared in patients with PCOS—hyperandrogenism and PCO morphology of the ovaries, which are clinically very important factors in decision-making during the treatment of infertility by medically assisted fertilization procedures. The role of androgens in folliculogenesis is still unclear and

there are conflicting results of studies dealing with this problem. The results of studies analyzing differences in treatment outcomes among defined PCOS phenotypes indicate a negative effect of hyperandrogenism in IVF procedures, and a higher incidence of complications later in pregnancy [16]. In patients with phenotype A and B, for every 1 pg./ml increase in free testosterone concentration, the proportion of clinically confirmed pregnancies decreases by 50–60% as well as the proportion of live births [17]. According to recent findings, the differences between PCOS phenotypes refer only to the number of good embryos for transfer, which is significantly higher in patients with hyperandrogenism and ovulation disorder, but without the typical PCO morphology of the ovaries (phenotype B). The proportion of biochemical and clinically confirmed pregnancies, as well as the number of couples with born children, do not differ significantly among phenotypically different PCOS patients [17, 18]. In addition, studies indicate that the proportion of clinically confirmed pregnancies, is significantly lower in women with PCOS phenotypes A, B and C compared to control patients [17]. The number of children born does not differ in different PCOS phenotypes. In some areas of the world, certain PCOS phenotypes have not been found at all, for example, there are no phenotypes B and C among Vietnamese women with PCOS [19]. Since the anti-Müller hormone (AMH) is often elevated in patients with PCOS, it has become a powerful factor that should have prognostic value in clinically assessing the outcome of treatment with medically assisted fertilization, however, it has been proven useful only in the group of patients with phenotype B. The proportion of clinically confirmed pregnancies and the proportion of babies born increases by 1.3 times for each 1 ng/ml serum AMH concentration increase [17].

4. PCOS phenotypes and the impact on oocytes and embryos quality

PCOS patients' oocytes quality can be associated with the hormonal and metabolic conditions, and therefore, consequently with the quality of the embryo. Poorer oocyte quality is part of the problem of subfertility in patients with PCOS. There is evidence that oocyte quality depends on PCOS phenotype and accompanying diseases and conditions that are more common in PCOS patients. Oocyte quality is defined by the morphology and morphology of associated structures, such as zona pellucida, cumulus oophorus, and corona radiata. An ovarian microenvironment in which follicles and oocytes grow and mature is exposed to multiple hormonal abnormalities in patients with PCOS. Well-known disruptive mechanisms include elevated concentrations of LH (luteinizing hormone) and FSH (follicle-stimulating hormone), impaired ratio of these hormones, elevated AMH values, impaired insulin-like growth factor secretion, and enzymes involved in the conversion of androgens to estrogens.

Hyperandrogenism interferes with the normal feedback loop between the ovaries, pituitary gland, and hypothalamus, which leads to an increased frequency of excretion of the releasing hormone for gonadotropins, and consecutively results in premature luteinization of granulose cells and abnormal maturation of the oocytes. There is also a direct effect of hyperandrogenism on the oocyte by activating its proapoptotic mechanism [20]. Hyperandogenic ovarium microenvironment interferes with the oocyte in the continuation of meiosis, promotes mitochondrial abnormalities and oxidative stress, and interferes with lipid metabolism in the oocyte [21].

High concentrations of AMH synthesized by granulosa cells, inhibit the recruitment of follicles, and therefore, the selection of follicles that will ovulate, leading to a vicious cycle of anovulation and hyperandrogenism. In addition, by blocking

the action of FSH on follicle growth and blocking the action of aromatase in charge of converting androgens synthesized in theca cells to estrogens in granulosa cells, the chronic state of hyperandrogenism is again supported. There is evidence that in patients with PCOS an increased concentration of AMH in follicular fluid exists along with oocytes of low quality. Molecular mechanisms that lead to disruption in the growth and maturation of oocytes are not known [22]. Significantly lower follicular fluid AMH levels were observed in follicles of fertilized MII oocytes than in non-fertilized non-PCOS patients [23]. Also in our non-PCOS patients with sterility and impaired fertility, gene for the AMH and androgen receptor in human cumulus cells surrounding morphologically highly graded oocytes are underexpressed [24].

Hyperinsulinemia, insulin resistance, and obesity are metabolic disorders associated with PCOS that intertwine with hormonal disorders and further worsen the conditions of oocyte microenvironments. Hyperinsulinemia reduces the synthesis of binding globulin for sex hormones (SHBG), and insulin also competes with androgens for binding sites on this carrier, which means that it promotes hyperandrogenism and all its negative effects. The direct effect of hyperinsulinemia on oocytes has been proven to disrupt the expression of genes associated with the dynamics of the division spindle and the function of centrosomes. In the case of insulin resistance, there is a change in gene expression for glucose carriers in granulose cells, and therefore, a possible decrease in energy sources for the metabolism of the oocyte itself and the processes of meiosis [25].

Based on PCOS phenotype in the population of women being treated with medically assisted reproduction procedures, no difference has been found so far in the proportion of oocytes in metaphase II, percentage of fertilization, or the evaluation of quality embryos for transfer [17, 26]. According to available data to date, patients who have a classic PCOS phenotype (A and B) associated with insulin resistance and obesity also have the highest risk for low-quality oocytes [27].

Besides poor quality oocytes, PCOS patients can have larger numbers of germinal vesicle stages – metaphase I oocyte collected from IVF, due to their elevated antral follicles count. Those are commonly maturated with unsatisfactory results. When optimized maturation procedure will serve, not only for PCOS and infertile patients but also in cancer patients for the preservation of fertility and as a more patient-friendly alternative than standard controlled ovarian stimulation. PCOS patients are not the only ones that could benefit from *in vitro* maturation (IVM) technology. IVM has numerous clinical applications. Under proper culture media additives, immature oocytes in the stage of metaphase I go to the final stage of maturation [28]. Although the IVM seems to have improved lately [29], still a success rate remains lower than traditional IVF [30]. International guidelines do not favor IVM over the other options due to lack of evidence [5] but conceived children are not endangered after IVM procedure [31]. Improving the IVM techniques can definitely increase the success of IVF/ICSI procedures in PCOS patients and lower the risk of OHSS.

5. Conclusion

The definition of phenotypes of polycystic ovarian syndrome stemmed from a diverse and complex clinical picture of this endocrine disorder. Diagnostic criteria of individual phenotype, contribute to new concepts of research into the effects of obesity, hyperandrogenism, and metabolic disorders on reproduction in humans. According to the outcomes of the treatment of infertility of patients with this disorder,

significant differences in the chances of conception compared to the population of infertile women who do not have polycystic ovary syndrome have been clearly proven. Less clear is the difference in infertility treatment outcomes between women with a defined polycystic ovarian syndrome phenotype, which is the area of new research. In cases of classical phenotype polycystic ovarian syndrome (A and B) associated with obesity and insulin resistance, negative effects of this disease on gametes and embryos are possible due to cellular process disorders related to glucose and androgen metabolism.

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Conflict of interest

Authors have no conflict of interest.

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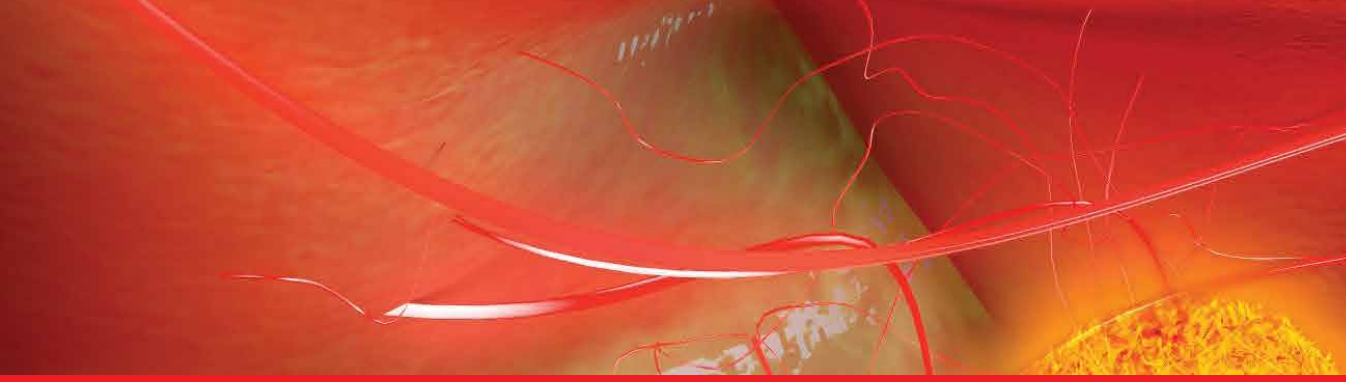

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Polycystic ovary syndrome (PCOS) is a heterogeneous hormone-imbalance disorder that occurs in reproductive-aged women worldwide and is characterized by hyperandrogenism, ovulatory process dysfunction and polycystic ovaries. This book includes two sections that cover the pathogenesis and treatment of PCOS. It provides a comprehensive overview of the latest PCOS research to benefit the population of women with this disorder.

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