Chapter 10
Oxidative Stress Impact on the Fertility of Women with Polycystic Ovary Syndrome

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Abstract Polycystic ovary syndrome is a common endocrine abnormality in reproductive-age women. The pathophysiology of this condition remains unclear. Women with polycystic ovary syndrome present a diverse combination of clinical complications including, psychological problems, reproductive alterations, and metabolic sequelae. In affected women, hyperglycemia, independent of obesity, promotes reactive oxygen species. The resultant oxidative stress causes extensive cellular injury, demonstrated by protein oxidation, lipid peroxidation, and DNA damage. This oxidative stress may directly stimulate hyperandrogenism. Additionally, serum total antioxidant status, is diminished in women with polycystic ovary syndrome, decreasing the body’s defense against an oxidative environment. Treatment through lifestyle intervention and medical/surgical therapy may improve metabolic consequences of polycystic ovary syndrome, including insulin resistance and reproductive status.

Keywords Oxidative stress impact · Fertility of women · Polycystic ovary syndrome · Metabolic syndrome · Psychological problems · Reproductive alterations · Metabolic sequelae

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10.1 Background

Polycystic ovary syndrome (PCOS) is a condition characterized by ovulatory dysfunction, hyperandrogenism, and polycystic ovaries. It is the most common endocrine abnormality in reproductive-aged women, with the Rotterdam Criteria [1] (See Fig. 10.1) showing the prevalence of PCOS at approximately 18%. Studies show that insulin resistance (IR) may be central to the etiology of the syndrome [2]. In IR, adipose, muscle, and liver cells do not respond appropriately to insulin, causing circulating glucose levels to remain high and leading to glucose intolerance and hyperinsulinemia [3].

Compensatory hyperinsulinemia causes decreased levels of sex hormone binding globulin (SHBG), a glycoprotein that binds testosterone and estradiol. This process increases the bioavailability of circulating androgens, which further stimulates increased androgen production in the adrenal gland and ovary [1]. Overweight or obesity affects 50–80% of women with this disorder and can aggravate IR and anovulation [4].

The current recommendation by Androgen Excess Society (AES) is to measure free testosterone for measuring circulating androgens. Both the adrenal glands and ovaries contribute to the concentration of circulating androgen in women. Still, the ovary is the primary source of testosterone, accounting for 75% of its circulating levels. The ovary produces androstenedione, which is converted in the liver, fat, and skin into testosterone. The adrenal glands secrete dehydroepiandrosterone (DHEAS), which may be elevated in PCOS. These hormones may act as precursors for more potent androgens such as testosterone and dihydrotestosterone. Their measurement may be useful in cases of rapid virilization [1].

The approach to diagnosis and treatment of PCOS is a challenge based on the complexity and diversity of its pathophysiology, including the association between metabolic syndrome and oxidative stress (OS) and their impacts on female fertility.

10.2 Clinical Manifestations

Menstrual disorders are among the most common signs of women with PCOS, ranging from amenorrhea to menorrhagia. In women with PCOS, abnormal menstruation is usually attributed to chronic anovulation, a steady state in which monthly rhythms associated with ovulation are not functional. This lack of ovulation creates fertility problems in these women.

Peripheral androgen excess manifests externally as an increase in acne and hirsutism. In hirsutism, hair is commonly seen on the upper lip, chin, around the nipples, and along the linea alba of the lower abdomen. Other signs of hyperandrogenism such as clitoromegaly, increased muscle mass, and voice deepening are more characteristic of an extreme form of PCOS termed hyperthecosis. Patients with PCOS may have dark, pigmented skin on the nape of their neck, skin folds,
knuckles, and/or elbows, called acanthosis nigricans, and is attributed to insulin resistance, or in some cases, to visceral malignancies such as stomach cancer [1]. Signs of IR such as hypertension, obesity, centripetal fat distribution, and acanthosis nigricans may predispose the patient to metabolic syndrome, nonalcoholic fatty liver [5], and obesity-related disorders. In turn, these conditions are risk factors for long-term metabolic sequelae like cardiovascular disease (CVD) and diabetes mellitus (DM) type 2. Approximately 10 % of women with PCOS have type 2 DM, and 30–40 % of women with PCOS have impaired glucose tolerance by the age of 40 [1].

Fig. 10.1 Recommended diagnostic criteria for polycystic ovary syndrome
Obesity is a comorbidity that may exacerbate the effects of PCOS. However, it is not a diagnostic criterion for PCOS, and nearly 20% of women with this condition are not obese [1]. Obesity itself increases hyperandrogenism, infertility, and pregnancy complications by aggravating PCOS. Along with IR, obesity may further augment the risks for developing DM type 2 and CVD.

10.3 Metabolic Syndrome

Numerous patients with PCOS have characteristics of metabolic syndrome. One study showed 43% prevalence of metabolic syndrome in women with PCOS [6].

Metabolic syndrome involves a clustering of hyperglycemia/IR, visceral obesity, and dyslipidemia. In 2001, the National Cholesterol Education Program Adult Treatment Panel III (2001) established a definition for the metabolic syndrome [3], which was renewed in 2005 by the American Heart Association (AHA) and the National Heart Lung and Blood Institute in 2005 [7]. According to this revised definition, metabolic syndrome requires the presence of three or more of the five criteria below [3] (See Table 10.1).

In 2005, The International Diabetes Foundation (IDF) published new criteria for metabolic syndrome [8]. It includes the same criteria as the other definitions, but requires obesity, as opposed to IR. The IDF definition has been challenged for its emphasis on obesity rather than IR, although visceral obesity is a known important factor [9]. In addition, the concurrence of obesity, oxidative stress, and platelet aggregation exponentially increases the risk of CVD morbidity and mortality [10, 11].

Mechanisms of metabolic syndrome are based on four clinical features:

A. Insulin Resistance
The pancreas produces insulin in response to increased levels of glucose. Subsequently, different tissues of the body such as the liver, adipose tissue, and skeletal muscle use this glucose as fuel. In adipose tissue, insulin prevents fat breakdown and promotes glucose uptake, while in skeletal muscle and the liver, it promotes glycogen synthesis and prevents glycogenolysis. In IR, these tissues do not respond adequately to insulin, which exacerbates hyperglycemia [12].

Physiological insulin signaling takes place after the binding of insulin to its receptor, a ligand-activated tyrosine kinase, resulting in tyrosine phosphorylation and activating two parallel pathways: the phosphoinositide 3-kinase (PI3 K) pathway and the mitogen-activated protein (MAP) kinase pathway [3]. The PI3 K is responsible for many of the metabolic effects of insulin including the activation of endothelial nitric oxide synthetase (eNOS). The MAP kinase pathway leads to vasoconstriction; growth effects on vascular smooth muscle cells; and expression of the vascular adhesion molecules VCAM-1 and E-selectin [3]. In IR the PI3 K pathway is affected and the MAP pathway is not. This misbalance results in a reduced nitric oxide (NO) formation, leading to endothelial dysfunction. A reduction also occurs in GLUT4, an insulin-responsive glucose transporter, translocation leading to decreased skeletal and fat glucose uptake [3].
B. Visceral adiposity

Visceral adiposity is closely associated with IR. Tumor necrosis factor α (TNF α) and interleukin6 (IL-6), inflammatory molecules produced by the adipose tissue, are thought to be closely involved in the pathogenesis of IR and vascular disturbance [13].

C. Dyslipidemia

Dyslipidemia comprises high levels of triglycerides, low-density lipoprotein (LDL), and low levels of high-density lipoprotein (HDL). IR leads to dyslipidemia mainly by preventing lipolysis, thereby increasing free fatty acid (FFA) concentration. In the liver, FFAs participate in the synthesis of triglycerides and in the formation of very low-density lipoprotein (VLDL) particles [14].

D. Endothelial dysfunction

The endothelium regulates physiological and pathological stimuli by maintaining homeostasis and preventing the development of atherosclerosis [12, 15, 16]. Endothelial dysfunction may result from the influence of FFAs, cytokines, hyperglycemia, or OS. IR and visceral adiposity are intimately related in promoting the damaging activity of the endothelium by increasing inflammatory reactants such as, diminishing blood flow to skeletal muscle, and further increasing reactive oxygen species (ROS). This creates a vicious cycle of endothelial dysfunction and formation of OS [3].

10.4 Polycystic Ovary Syndrome, Metabolic Syndrome, and Oxidative Stress

Oxidative Stress occurs (OS) when the production of ROS exceeds the endogenous anti-oxidant defense. The anti-oxidant status of plasma suggests the extent of both OS and anti-oxidant levels. A moderate increase in ROS can promote cell growth and proliferation. On the contrary, excessive ROS accumulation will result in cellular injury, such as damage to DNA, protein, and lipid membranes. This is why PCOS is considered an oxidative state. In women with PCOS, diminished total antioxidant capacity (TAC) is the sum of concentration of individual antioxidants such as thiol, carotene, Vitamin C and E, which leads to a decrease in antioxidants defense.

Metabolic IR may occur due to endothelial nitric oxide (NO) impairment. NO is a regulator of important nervous, immune and cardiovascular processes such as arterial vasodilation; it increases blood flow and promotes vascular smooth muscle relaxation. In both obesity and PCOS, stimulation of muscle by insulin is inhibited by a defect in endothelial synthesis [17, 18].
Markers of OS have been proposed to increase exponentially between partial and full metabolic syndrome during childhood and puberty. 15-F2t-Isoprostane is a marker for OS in humans [19, 20] and is synthesized non-enzymatically. Within the cell membrane, this process takes place through the impact of free radicals on the arachnoid acids of phospholipids and plasma lipoproteins, culminating in lipid peroxidation [21].

Protein carbonyl content, a marker for protein oxidation and another important indicator for OS, was found to increase gradually during the progression from partial to full metabolic syndrome in childhood. Attack by hydrogen peroxide (H$_2$O$_2$) or oxygen (O$_2$) can facilitate redox cycling cations such as Fe$^+$ and Cu$^{2+}$ to attach to binding sites on proteins. Some amino acid residues are converted to carbonyl derivatives and as a result of oxidative modification, these proteins become highly susceptible to degradation. This oxidative modification is an early marker of oxygen radical-mediated tissue damage [22]. In addition to these OS markers, the concentration of tromboxane (TXB2), a potent vasoconstrictor produced by lipid peroxidation, substantially increases the risks for the development of atherosclerosis and other precursors of CVD [10, 11] (See Fig. 10.2).

Acute hyperglycemia causes an increase in the generation of ROS from mononuclear cells (MNC). MNC’s promote the release of tumor necrosis factor (TNF) $\alpha$, a known mediator of IR, and activate the proinflammatory transcription factor nuclear factor (NFkB), which further increases TNF$\alpha$ concentration [23]. MNCs of women with PCOS are in a proinflammatory state as evidenced by their increased sensitivity to physiologic hyperglycemia and elevated C-reactive protein, a marker of inflammation. ROS generation is directly related to androgen levels, which can explain why OS in response to hyperglycemia may be capable of directly stimulating hyperandrogenism [23].

The mitochondria are one of the main sites for ROS production by peripheral blood leukocytes [24]. IR in PCOS patients disrupts mitochondrial function, leading to increased ROS production, a reduction in glutathione levels and decreased oxygen consumption by the mitochondria [25].

Homocysteine is an intermediate formed during disruption of the amino acid methionine, or during trans-sulfuration to cystathione and cysteine. Homocysteine

![Fig. 10.2 Risk factors for metabolic syndrome](image-url)
is closely implicated in the risk of developing CVD and other complications, by promoting OS in vascular endothelium, activation of platelets, blockage of blood flow, and stimulation of vascular smooth muscle proliferation. Homocysteine affects endometrial blood flow and vascular structure, thereby impairing adequate implantation. Both impaired implantation and increased rates of miscarriage might be due in part to elevated homocysteine and are more frequent in PCOS, even after controlling for ovulatory abnormalities, increased LH, and hyperandrogenism [26]. Increased homocysteine exacerbates some of the long-term complications of metabolic syndrome such as DM, hypertension, nephropathy, dyslipidemia, and CVD; IR alone is a risk factor for all of these complications. In metabolic disorders, PCOS may conceivably be considered an early indicator of IR syndrome (IRS).

The preovulatory follicle, which is a metabolically active environment, is likely to have multiple sources of ROS production, making it susceptible to OS. The follicular fluid (FF) environment is composed of the oocyte, granulosa cells, and surrounding cells, such as endothelial and theca cells. FF contains cytokines, neutrophils, and macrophages, all of which can participate in the production of oxygen free radicals. Monooxygenase reaction, required for the steroidogenic process and mediated by cytochrome P450, invariably results in ROS production [27]. For better reproductive results in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles, certain cutoff levels of ROS in FF are needed for pregnancy. On the other hand, excessive ROS levels in the FF lead to toxic effects.

Superoxide radical is a free radical that is produced by the activity of NADPH oxidase. Lean women with PCOS showed elevated ROS generation and p47phox, a key protein component of NADPH oxidase. ROS production parallels androgen levels, while p47phox is inversely correlated to insulin sensitivity. The association among p47phox protein expression and percent visceral fat implies that elevated abdominal adiposity may be a key factor of the ROS-induced OS and a promoter of insulin action as seen in obese women with PCOS [23]. This suggests that ROS-induced OS may play a role in the associated development of IR and hyperandrogenism.

Another important consideration is the presence or absence of meiotic spindle (MS) in oocytes. The presence of MS is associated with lower OS and has improved intracytoplasmic sperm injection (ICSI) outcome both in women with PCOS and tubal factor infertility. However, in the absence of MS, there is significant reduction in the formation of good quality embryo and fertilization rate, though statistically not significant, is decreased in PCOS women [27]. Glutathione, an enzymatic antioxidant, plays an important role in maintaining MS morphology and in early blastocyst stage development [28].

A larger number of preantral follicles occur in PCOS ovaries. This disorderly growth takes place due to impaired apoptosis at an early stage of follicular growth, promoting many follicles to synthesize androgens in their thecal component. ROS initiates the apoptotic cascade in granulosa cells, while antioxidants antagonize these effects on cultured preovulatory follicles [29–31]. Free radicals can activate
meiosis in immature oocytes, which is inhibited by antioxidants. Antioxidants block gonadotropin-induced oocyte maturation in follicles and oxygen radicals promote the activations of meiosis. This process may explain the persistence of immature follicles in PCOS [32]. Therefore, oxygen radicals may be categorized as potential mediators for the resumption of meiosis in the oocyte, which is activated by the ovulatory surge of LH.

TAC has been known to promote cell proliferation on ovaries. Development of basement membrane during follicular growth is possible due to adequate collagen synthesis, which is attained from antioxidant functions [31]. Antioxidants are encountered in tissues where steroid hormones are produced and maintain adequate control to ensure that steroidogenesis takes place despite the occurrence of lipid peroxidation [33]. Antioxidants, particularly vitamin E, have been shown to promote LH release from the pituitary gland upon activation by gonadotropin secretion [34].

OS and anti-oxidant status is universally in all PCOS patients, including those who are lean and without metabolic disturbances [35] (See Fig. 10.3).
10.5 Treatment

Women with PCOS who are not attempting to conceive:

A. Lifestyle modifications

Exercise of 30 minutes 3 times a week and a calorie-restricted diet are key factors shown to reduce DM and cardiovascular risk [36]. Even a 5–10 % weight loss has been found to lower androgen levels, decrease hirsutism, and reduce cardiovascular morbidity and mortality. In addition, this weight loss has been shown to increase SHBG, improve glucose and lipid levels, and promote spontaneous resumption of menses [37–40].

B. Combined oral contraceptives

Combination low-dose hormonal contraceptives are most commonly used for long-term management and are considered the primary treatment of menstrual disorders. They offer benefit by suppressing LH levels, ovarian androgen secretion, and increasing SHBG [1].

Oral contraceptives (OCPs) improve circulating markers of endothelial function. Estrogen contained in OCPs improved markers of endothelial function which counteracts the negative effects of impaired IR on endothelial activity [41]. There is no evidence that proves that women with PCOS who use OCPs suffer more cardiovascular events than the general population does [42, 43].

C. Insulin sensitizing agents

Insulin sensitizing agents improve peripheral insulin sensitivity by decreasing circulating androgen levels as well as improving ovulation rate and glucose tolerance. Studies have focused on agents that alleviate insulin sensitivity, including biguanides (e.g., metformin) and thiazolidinediones (e.g., rosiglitazone) [44, 45]. Thiazolidinediones have been unsatisfactory among PCOS patients due to increased weight gain.

Metformin reduces a significant number of risk factors for type 2 DM and CVD, including IR, inflammatory markers, and circulating markers of endothelial function in women with PCOS. This suggests an indirect association between IR and endothelial function and provides new understanding into the relationships between them [41].

A long-term study suggested that metformin continued to improve metabolic complications in women with PCOS over a 36-month treatment course, with particular improvement in circulating HDL (HDL-C), diastolic blood pressure, and BMI [46]. However, current data is insufficient to safely recommend metformin to all women with PCOS.

10.6 Conclusion and What’s Next

OS and anti-oxidant status is omnipresent in all PCOS patients, including those who are lean and without metabolic disturbances. ROS generation from MNCs in response to hyperglycemia may serve as an inflammatory trigger for the induction
of IR in PCOS. The resultant OS induces a proinflammatory state that may further contribute to IR and hyperandrogenism in PCOS. This IR increases risk for DM2 and several metabolic abnormalities that predispose patients to CVD.

Further study on OS defenses in PCOS will provide a better understanding into the mechanisms that result in decline of fertility. The in vitro maturation (IVM) technique has been used as an alternative treatment for PCOS-related infertility, however the clinical results of IVM have not been encouraging [47, 48]. As IVM does not require priming with FSH or hCG, it possesses several advantages for women with PCOS such as avoiding ovarian hyperstimulation [49]. Future research on PCOS needs to focus on improving the IVM technique to achieve high embryo implantation rates. Currently, new research emphasizes a familial basis for PCOS and the related pattern of diabetes, which may unveil the true nature of insulin resistance.

References