

## Chapter 8: Nanotechnology-Driven Antioxidants and Immunomodulators for Polycystic Ovary Syndrome (PCOS) Therapy

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### Abstract

PCOS is a complex disorder of the endocrine system characterized by the presence of multiple cysts within the ovaries, affecting women of reproductive age, and also involving chronic, low-grade inflammation, oxidative stress, and problems with immunological regulation, among others. It is true that the traditional treatment of PCOS focuses on the hormonal dysregulation and imbalance, but remains a blind spot the inflammation that contributes to the dysregulation of the metabolic and reproductive systems. The use of nano immunology and, specifically nano-therapy, focuses on the development of nano-antioxidants and other immuno-modulatory agents. They offer unique and new opportunities for PCOS treatment. This chapter describes the inflammation- PCOS linkage from a mechanistic perspective and elaborates on the ovarian inflammation caused by excess reactive oxygen species and immune cells. It also describes how engineered nano-antioxidants are able to remove excess reactive oxygen species and restore the redox balance and immune modulation, and describes the clinical proposal of immuno-modulators, chemical and natural, to diminish PCOS systemic and local inflammation. There is a strong emphasis on the ability and the promise that systems of nanotechnology offer for the postulation of PCOS treatment aimed on the inflammation and dysregulation of the immune system.

**Keywords:** *PCOS, inflammation, nano-antioxidants, immuno-modulators, oxidative stress, nanomedicine, ovarian dysfunction, targeted therapy, ROS*

## 1. INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is the leading metabolic and endocrine disorder among women of reproductive age that accounts for around 6, 15% of the population worldwide depending on the diagnostic criteria used. Initially, it was reported to be the triad of Oligo/amenorrhea, Hyperandrogenism, and Polycystic ovaries morphology, and now it is known as a systemic disorder with inflammatory and oxidative stress features besides the others as well. PCOs is characterized by a special type of chronic inflammation that includes elevated levels of C, reactive protein (CRP), Tumor necrosis factor, alpha (TNF, ), Interleukin, 6 (IL, 6), and other pro, inflammatory agents. The inflammation and stress that result from this condition worsen insulin resistance and hyperandrogenism and also increase oxidative stress in a vicious circle that eventually leads to ovulation dysfunction, folliculogenesis disruption, and increased cardiometabolic risk in the long term.

The existing treatment strategies mainly focus on the control of symptoms which include menstrual cycle regulation, metabolic disorder control, and management of hyperandrogenism and the metabolic aspects of the disorder. Very few indirectly affect inflammation and stress. To this extent, antioxidants and immune, modulatory agents, particularly those that employ nanotechnology, are new promising approaches. Nanoformulations enhance natural and synthetic compounds' bioavailability, stability, and tissue, specific delivery, whereas immuno, modulators help in restoring immune homeostasis and reduce pathogenic inflammatory and stress. To this extent, antioxidants and immune, modulatory agents, particularly those that employ nanotechnology, are new promising approaches. Nanoformulations enhance natural and synthetic compounds' bioavailability, stability, and tissue, specific delivery, whereas immuno, modulators help in restoring immune homeostasis and reduce pathogenic inflammatory

### **Epidemiology and Clinical Manifestations**

PCOS is often regarded as the most prevalent endocrine metabolic disorder in women of reproductive age, being estimated to have a worldwide prevalence of 6% to 15% depending on applied diagnostic criteria. The NIH 1990 criteria are quite limited as they focus on chronic anovulation and hyperandrogenism solely, while the Rotterdam 2003 criteria are wider as they allow for the diagnosis to be based on any two of three presenting features: oligo/anovulation, hyperandrogenism, and polycystic ovarian morphology on ultrasound. The AE-PCOS Society 2006 criteria indicate hyperandrogenism as a critical diagnostic aspect. As a result, the different prevalence rates among studies are typically due to the Rotterdam criteria giving higher estimates. The ethnic and regional differences are very pronounced South Asian, Middle Eastern, and Hispanic women are often found to have higher prevalence rates, more serious metabolic disorders, and earlier symptoms compared to Caucasian women. The

disorder usually becomes manifested during teenage years, just after the start of menstruation, but the diagnosis can take time because of the similarity between PCOS symptoms and normal adolescent body changes. Among risk factors are genetic make-up, intrauterine exposure to androgens, being overweight and living a sedentary life, and food habits with family clustering indicating a sizable heritable component.

The signs appearing due to PCOS are not uniform for all patients. The symptoms encompass reproduction, androgen excess, metabolic changes, and psychological factors, and they can vary a lot not only between individuals but also within the same person over time. Menstrual and related symptoms are the largest group, these being oligomenorrhea, amenorrhea, and chronic anovulation, often resulting in infertility or subfertility. Moreover, there are also women with polycystic ovarian morphology who have at least 12 small follicles (< 10 mm) and whose ovaries have an increased volume (>10 mL) according to the ultrasound. The signs of hyperandrogenism show up in the skin signs such as hirsutism, acne, and androgenic alopecia which can be strong in the case of the patient and have a deep impact psychologically. Metabolic changes are also very common in this condition with insulin resistance showing up in both the heavy and the light phenotypes, sometimes the central obesity, dyslipidemia (high triglycerides and low HDL cholesterol), and the increased risk for type 2 diabetes mellitus and metabolic syndrome coming along with it. These changes in the metabolism are the cause of the high long-term risk of cardiovascular disease.

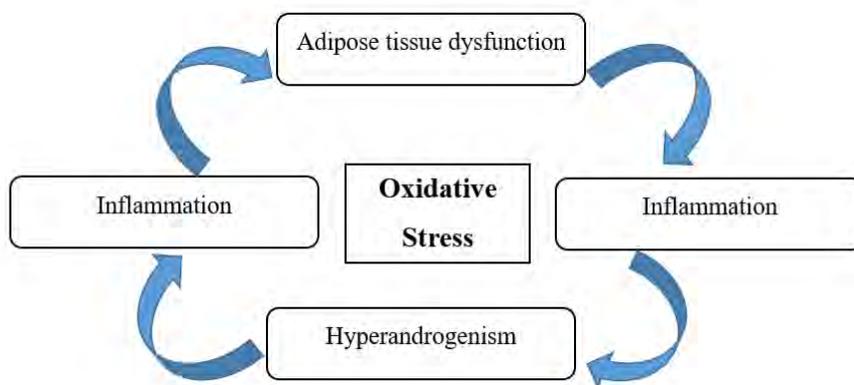
Psychological problems are another area where PCOS impacts patients significantly in a negative way, i.e., moods such as depression and anxiety, unconfidence, and body image discontent. Unhealthy eating practices are common, especially among and young women, and may further increase the metabolic risk. In addition, women with PCOS will experience infertility and the development of disease conditions such as endometrial hyperplasia and carcinoma due to prolonged unopposed estrogen exposure, as well as non-alcoholic fatty liver disease (NAFLD) and obstructive sleep apnea, especially in the case of obesity [4-6].

### **Role of Inflammation and Oxidative Stress in PCOS Pathogenesis**

PCOS was previously treated as a matter of hormones or metabolism. It has now become widely known that it is a condition marked by chronic low, grade inflammation and continuous oxidative stress. These two occurrences are, in fact, very closely linked to each other, which not only triggers PCOS to a great extent but also worsens the condition over time. They found that the inflammatory markers tumor necrosis factor, alpha (TNF, ), interleukin, 6 (IL, 6), interleukin, 18 (IL, 18), and C, reactive protein (CRP) were significantly elevated in women with PCOS as compared to healthy women. The situation is even more dramatic in the case where obesity is not present at all. The state of chronic inflammation that has persisted up to now is mainly due to the interaction of the following causes: genetic predisposition, adipose tissue (fat)

dysfunction, insulin resistance, and hormone imbalance. One of the major reasons for this inflammation is the malfunction of visceral fat. In PCOS, the fat cells often become hypertrophic and thus increase the secretion of leptin and resistin, which are adipocyte, derived inflammatory mediators, while they decrease the secretion of the anti, inflammatory adipokine adiponectin. The pro, inflammatory environment that results from this attracts macrophages and other immune cells to the fat pads, which in turn release inflammatory cytokines as well.

Oxidative stress refers to the situation when the body generates more reactive oxygen species (ROS) than the antioxidant defense system will be able to neutralize, adds another dimension of injury. Individually, the overproduction of ROS in PCOS is disruptive to the cell structures reaching out to lipids, proteins and DNA in the ovaries. The damage to the granulosa cells, which are the most essential cells for the maturation of the egg, and the stoppage of normal follicle growth can ensue due to the impaired function of the cells. In addition, the activation of inflammatory pathways, such as nuclear factor, kappa B (NF, B), due to oxidative stress, thus, upregulates the inflammatory molecules production, is the other effect of this phenomenon. Mitochondrial dysfunction frequently seen in PCOS scenario, amplifies the problem by increasing ROS production as well as, by disrupting cellular energy balance, which in turn results in the lowering of egg quality. Inflammation and oxidative stress thus co, exist in a loop that is self, perpetuating: cytokine, induced ROS production enhances oxidative stress, whereas, oxidative stress is responsible for the release of more inflammatory signals. This vicious circle does not only affect reproductive health; it, also, elevates the risk of systemic complications such as, damage to blood vessels, atherosclerosis, and metabolic syndrome. The knowledge of this connection is essential since the management of both inflammation and oxidative stress simultaneously might not only contribute to the improvement of fertility but also facilitate the preservation of cardiovascular and metabolic health in the long run in women with PCOS.



**Figure 8.1.** Roles of Inflammation and oxidative Stress in PCOS Pathogenesis

## 2. PATHOPHYSIOLOGICAL ROLE OF IMMUNE DYSREGULATION IN PCOS

PCOS has been progressively acknowledged as an endocrine disorder as well as a metabolic issue, minor inflammation, and immune malfunction which were mainly the causes of the latter. Specifically in PCOS, the body's natural and adaptive immunity are modified to be in a continuous pro-inflammatory state this state is one of the factors causing the anomalies in both the reproductive and metabolic areas. In women with the disease, pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), and interleukin-18 (IL-18) are present at higher levels than normal. Such cytokines activate intracellular signaling pathways like NF- $\kappa$ B, which in turn, continued the vicious cycle of oxidative stress, insulin resistance, and ovarian dysfunction. In PCOS, macrophages, dendritic cells, and natural killer (NK) cells not only show increased numbers but also the signs of activation which their activity then results in the release of inflammatory mediators and recruiting more immune cells into the ovary and thus inflaming the organ. The inflammation in the ovary hampers ovulation and cyst formation by disrupting folliculogenesis. Furthermore, one-way immune alterations are modification of T-helper (Th) cell subsets (e.g., increased Th1/Th17 responses and reduced Tregs), which together with other factors, don't allow the ovary to be immunologically tolerant. Because of the immune dysfunction that characterizes PCOS, the adipose tissue is also affected as activated immune cells release of adipokines like leptin and resistin that cause the inflammation in the body to be more systemic and worsen insulin resistance. The interaction among the immune cells, adipocytes, and ovaries sets up the whole process of maintaining inflammation live. The resulting combination of oxidative stress, immune imbalance, and metabolic dysfunction not only drives the core features of PCOS hyperandrogenism, menstrual irregularities, and polycystic ovarian morphology but also heightens the risk for long-term complications like type 2 diabetes mellitus, cardiovascular disease, and infertility. (11,12,13)

### Cytokine Imbalance

Cytokine imbalance is a key feature of the chronic low-grade inflammation seen in polycystic ovary syndrome (PCOS) and plays a pivotal role in its development. Women with PCOS often have persistently higher levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), interleukin-1 $\beta$  (IL-1 $\beta$ ), and interleukin-18 (IL-18) alongside reduced levels or activity of anti-inflammatory cytokines like interleukin-10 (IL-10) and transforming growth factor-beta (TGF- $\beta$ ). This shift toward a pro-inflammatory state activates key signaling pathways, including NF- $\kappa$ B and JAK/STAT, which further worsen insulin resistance, oxidative stress, and excess androgen production. In the ovaries, these elevated inflammatory cytokines

disrupt the normal activity of granulosa and theca cells. This can impair follicle maturation, trigger premature luteinisation, and stimulate theca cells to produce more androgens, aggravating hyperandrogenism. High IL-18 levels, in particular, have been linked to metabolic disturbances, creating a bridge between systemic inflammation and cardiovascular risks in PCOS. Obesity and excess visceral fat common in PCOS add to the problem by producing inflammatory adipokines that amplify cytokine release. The result is a self-perpetuating inflammatory loop that not only drives hormonal and reproductive issues but also increases the risk of long-term complications like type 2 diabetes, abnormal lipid profiles, and atherosclerosis. Addressing this cytokine imbalance offers a promising avenue for new therapies. Emerging strategies, including nano-antioxidants and immune-modulating agents, hold potential for restoring immune balance, reducing inflammation, and improving both reproductive and metabolic outcomes in women with PCOS. (14,15,16)

### **Innate and Adaptive Immune Cells in Ovarian Tissue**

In PCOS, the continuous presence of both innate and adaptive immune cells in the ovarian tissue is a major factor in the development of the chronic inflammation that causes the disruption of the reproductive and metabolic processes. The ovary is rich in immune cells and among them are macrophages, dendritic cells, natural killer (NK) cells, T cells, and B cells, which together are responsible for the normal follicle growth, ovulation, and tissue remodeling. However, in the case of PCOS, the immune cells that are affected by the distribution, activation status, and functional balance of these immune cells become dysregulated, leading to pathological consequences. The innate immune cells, more specifically the macrophages are considerably present in the ovarian stroma and theca of the patients suffering from PCOS. These macrophages are shifting to a pro-inflammatory M1 type and producing a lot of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 that enhance the androgen production and hinder the follicle maturation. The number of NK cells in the ovary is lower than that of the uterus but they still show increased cytotoxic activity in PCOS which leads to follicular atresia and tissue destruction. Dendritic cells, which are the antigen-presenting cells, are hyperactivated and this results in T-cell-mediated inflammation becoming stronger and immune activation lasting longer.

The alteration of immune cells in the adaptive immune system occurs in PCOS-affected ovaries. Among the different types of immune cells, an increase of CD4+ helper T cells especially the pro-inflammatory Th1 and Th17 subpopulations seems to be the most pronounced. This increase in T-cell subpopulations produces the cytokines interferon-gamma and IL-17, which, respectively, further inflame and deteriorate the ovarian stroma. Regulatory T-cells (Tregs), those that usually prevent the overactivation of the immune system and maintain tolerance, have become less efficient or fewer, thus giving the anti-inflammatory control a harder time. Although B-

lymphocytes are less researched, they might be a factor in developing PCOS by their secretion of autoantibodies and pro-inflammatory cytokines that in turn cause the ovarian function to deteriorate. The communication between the innate and adaptive immune systems is a contributor to ovarian inflammation through the recruitment of macrophages and dendritic cells that activate T cells, and again the cycle is continued with the T cells releasing cytokines that attract more innate cells. The result is a continuously activated immune defense that prevents steroid hormones from being produced, inhibits ovulation, and brings about the development of multiple, immature cystic follicles. Immunotherapy built on these immune cell imbalances such as nano-antioxidants, cytokine blockers, and tolerance inducer agents are predicted to help in bringing back the ovarian immune balance and the resulting positive fertility outcome in PCOS. (17,18,19)

### **3. OXIDATIVE STRESS AND ROS IN PCOS**

Oxidative stress (OS) is widely recognized as a principal pathological mechanism in polycystic ovary syndrome (PCOS), and it is defined as the condition in which the levels of reactive oxygen species (ROS) exceed the ability of the body's antioxidant defense systems to neutralize them. Normally, in a healthy body, ROS, for instance, superoxide anion ( $O_2^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radicals ( $\bullet OH$ ), contribute positively to folliculogenesis, steroidogenesis, and ovulation by mineralizing these processes via secondary messengers in the cellular signaling pathways. On the contrary, in PCOS, the problem stems from the excessive production of ROS combined with the lowered capability of antioxidants, thus creating a pro-oxidant environment and consequently cellular and molecular damage. A combination of factors results in a high ROS level in PCOS, among which are chronic low-grade inflammation, hyperandrogenism, insulin resistance, and overweight-related metabolic stress. The release of pro-inflammatory cytokines like TNF- $\alpha$  and IL-6 causes the deterioration of the mitochondria in the ovaries and other tissues related to the periphery, thus making the electron transport chain lose even more ROS due to the higher rate of dysfunction. Insulin resistance leads to the formation of advanced glycation end products (AGEs), which, in turn, promote ROS generation through the activation of NADPH oxidase. Moreover, the dysfunctional fatty tissue in PCOS results in the liberation of more free fatty acids thereby intensifying the oxidative stress.

The pathological impacts of oxidative stress in PCOS are various and greatly contribute to the dysfunction of both the reproductive and metabolic systems. The overproduction of reactive oxygen species (ROS) results in ovarian dysfunction due to the damaging effects on granulosa and theca cells which leads to the neglect of estrogen production, thus, promoting follicle arrest and anovulation. At the same time, endothelial dysfunction is brought about since

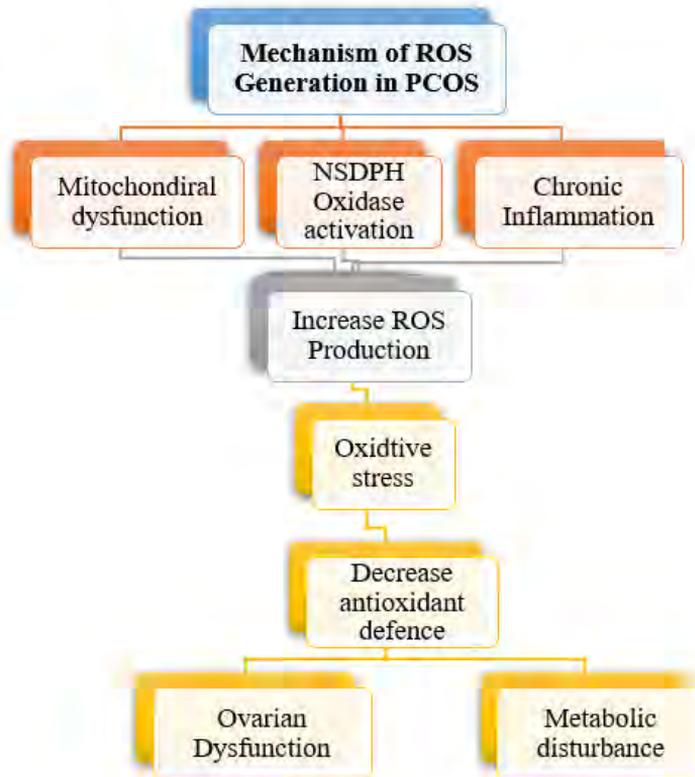
ROS decreases nitric oxide (NO) availability; consequently, vasodilation is impaired, and the risk of cardiovascular problems is increased. Moreover, the oxidative stress that has resulted is the cause of the cellular and protein destruction; among others, it is the ROs that are altering the nuclear and mitochondrial DNA and the key enzymes, which are responsible for the hormone production, thus eventually breaking down the normal cellular metabolism. Not only that, but also, oxidative stress is one of the main factors that lead to the emergence of insulin resistance as it forces the oxidation of the important signaling proteins in the PI3K/Akt pathway and this results in defective insulin receptor signaling and aggravation of the hyperglycemia that is most often seen in PCOS.

Besides, the evidence has pointed out a two-way relationship between oxidative stress and inflammation in PCOS. The ROS activate the redox-sensitive transcription factors such as NF- $\kappa$ B, which, in turn, increase the expression of genes that are pro-inflammatory and are thus being and the inflammation is producing the ROS further - this cycle that perpetuates the ovarian and systemic pathology is a vicious one. In the clinical setting, the PCOS women quite often exhibit high levels of malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG), which are oxidative stress markers, and are also found having low levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The biochemical profile of the patient is the basis of antioxidant strategies, still, the doctor should apply lifestyle changes and give the patient dietary polyphenols, vitamins C and E, N-acetylcysteine to reduce the ROS burden and for redox homeostasis restoration. (20,21,22)

### **Mechanisms of ROS Generation in PCOS**

In the case of PCOS, the formation of reactive oxygen species (ROS) is done through several linked chemical and cellular pathways, which together are one of the main factors for the disease's pathology. One of the biggest contributors to ROS is the impaired function of mitochondria in ovarian, adipose, and skeletal muscle tissues. In the insulin-resistant condition typical for PCOS, high blood sugar levels and increased free fatty acids are responsible for the upsurge in mitochondrial electron transport chain (ETC) activity. This increased activity results in the leaking of electrons, especially at complexes I and III, and this, in turn, leads to the partial reduction of oxygen into the formation of superoxide anions ( $O_2^{\bullet-}$ ). A seemingly next point to consider from the above discussion is the activation of membrane-associated NADPH oxidases (NOX enzymes), especially NOX4 and NOX2, which get activated by the combination of hyperinsulinemia, increased androgens, and low-grade chronic inflammation. By doing so, these enzymes help in building up the super oxide

component directly by drawing electrons from NADPH and feeding them into molecular oxygen. The pro-inflammation situation in PCOS, possibly characterized by high levels of cytokines such as TNF- $\alpha$  and IL-6, and the subsequent activation of immune cells (macrophages, neutrophils) increasingly produce ROS through the respiratory burst. Besides that, hormonal changes also add to the already increased level of ROS. Hyperandrogenism changes the process of ovarian steroid synthesis, resulting in progressive theca cell metabolism with more oxidative activity. Plus, there is a class of compounds known as advanced glycation end products (AGEs), which are formed when sugar binds to a protein or lipid, and their levels are found to be heightened in PCOS due to glucose being poorly controlled. RAGE is the receptor that is formed in the cells of the body and binds to AGEs and activates intracellular signaling pathways like NF- $\kappa$ B and MAPK, thus leading to ROS production. Finally, the downregulation of antioxidant-defense mechanisms like glutathione, superoxide dismutase (SOD), catalase, and glutathione peroxidase as well as reduced access to such defense mechanisms create an environment for oxidative stress to prevail.



**Figure 8.2** Mechanism of ROS Generation in PCOS

## Impact on Oocyte Quality and Ovulation

The combination of low-grade inflammation and oxidative stress (OS) in PCOS has a negative impact on the microenvironment of the ovary and thus on the quality of oocytes and fertilization. The high levels of reactive oxygen species (ROS) disturb the balance of oxidants and antioxidants that is, in fact, very delicate, thus causing at the same time lipid peroxidation, protein oxidation, and DNA fragmentation in the oocytes. The oxidative damage leads to the impairment of the mitochondrial function, the reduction of ATP generation, and the disturbance of meiotic spindle integrity, which is the reason why there is a higher risk of chromosomal misalignment and aneuploidy. By the activity of inflammatory cytokines like TNF- $\alpha$ , IL-6, and IL-1 $\beta$  the effects are pronounced even more because the granulosa and theca cell functions are altered, the production of estradiol is impaired, and the apoptosis of granulosa cells is promoted. All these changes slow down follicular maturation, cumulus–oocyte complex enlargement, and LH-triggered ovulation. Thus, there is a high occurrence of follicular arrest, failure of ovulation, and luteinization defects among women suffering from PCOS. From the clinic's perspective this means irregular menstrual cycles, reduced fertilization potential, and less than optimal ART outcomes. Restoring normal folliculogenesis, improving oocyte quality, and increasing the reproductive success rate of women with PCOS might be achieved through the addressing of oxidative stress and inflammatory pathways by means of targeted interventions, such as antioxidant supplementation, lifestyle change, and immunomodulatory therapy. (25,26)

## 5. NANO-ANTIOXIDANTS: A NOVEL STRATEGY FOR REDOX MODULATION

Nano-antioxidants are characterized by the use of antioxidant compounds (natural polyphenols, low-molecular-weight antioxidants, enzymes, or redox-active metal/metal-oxide species) in combination with nanoscale carriers (liposomes, polymeric nanoparticles, solid-lipid NPs, nanoemulsions, nanocrystals, metal/selenium NPs, etc.) to enhance their stability, biological half-life, tissue targeting, and intracellular delivery. The non-activated oxidative stress situation and active state in PCOS condition affect ovarian tissues and cells making the use of nano-antioxidants quite soothing because they effectively locate and direct the dispatch of antioxidant activity where it is urgently needed, thus inducing the said benefits of reduced side effects, and releasing in a controlled manner over time sustaining redox steady state. The relevance of the above has recently been further confirmed by the reviews and experiments where a number of different nanocarriers containing Curcumin, resveratrol, quercetin, coenzyme Q10, selenium, and other antioxidants tested on PCOS models have all been positive. (27)

## Types and Mechanisms of Nano-antioxidants

Nanocarrier systems which are commonly used in PCOS treatments: their advantages in terms of functionality

- Polymeric nanoparticles (PLGA, chitosan, alginate): shield unstable polyphenols against degradation, provide prolonged release, and can be impregnated with selective ligands for targeting.
- Lipid-based systems (liposomes, solid-lipid nanoparticles, nanoemulsions): not only boost but also facilitate oral uptake of fat-soluble antioxidants (e.g., curcumin, resveratrol) by their improved solubility.
- Metal/metal-oxide and selenium nanoparticles (Ag, ZnO, Se NPs): are endowed by nature with redox activity and can function as ROS scavengers with low-dose applications.
- Nanocrystals / nanosuspensions: Not only that but also increase dissolution rate and oral bioavailability of poorly soluble antioxidants.

The mentioned classes of carriers have been extensively utilized for the antioxidant types such as curcumin, resveratrol, quercetin, CoQ10, NAC derivatives, which are often referred to as poor solubility, low bioavailability, and rapid metabolism problems.

### Mechanisms of action (how nano-antioxidants blunt ROS and inflammation in PCOS)

- 52. Enhanced bioavailability and tissue delivery:** The process of nanosizing and encapsulation together leads to a significant increase in the plasma levels and the uptake of polyphenols by cells, thus resulting in a higher concentration of these compounds in the ovaries and fat tissues where the reactive oxygen species (ROS) are produced.
- 53. Sustained/controlled release:** The controlled release allows the gradual and continuous release of the antioxidant activity throughout the treatment period, thus preventing the peaks and troughs in exposure that usually result in limited efficacy.
- 54. Targeted intracellular action:** A few nanocarriers not only facilitate the escape from the endosome but also deliver the compound directly to the mitochondria thus allowing the consequent neutralization of the mitochondrial ROS production and the restoration of the ATP/mitochondrial function.
- 55. Multi-modal activity:** The antioxidants that are in nanoform can perform multiple actions at the same time by scavenging the free radicals, chelating the transition metals, inhibiting the NADPH oxidase (NOX) activation, and

downregulating the redox-sensitive inflammatory pathways (NF- $\kappa$ B, NLRP3 inflammasome) among others. Curcumin-loaded NPs, for instance, provide a blockade to TLR4/MyD88/NF- $\kappa$ B signaling in the case of PCOS models.

**56. Adjuvant immune modulation:** By the simultaneous reduction of the levels of ROS and the cytokines signalling, the nano-antioxidants are working at an indirect level to restore the balance of the immune cells (in the case of Treg activity being favored and M1 macrophage polarization being reduced) and to enhance the insulin signalling (PI3K/Akt) thus tackling the inflammation-insulin resistance loop which is at the very core of the PCOS pathogenesis. (27,28,29-32)

### **Safety, regulatory and practical considerations**

The biologic distribution and toxicity profiles can be modified by nano formulations: metallic NPs (Ag, ZnO) and certain surface treatments are prone to the risk of accumulation and organ toxicity, thus complete toxicological testing is essential. Polymeric and lipid-based delivery systems typically exhibit greater toxicity tolerance but necessitate standardization regarding their size, surface charge, and release rate. Long-term reproductive safety (effects on embryo/fetus) is particularly important in PCOS clinical studies and demands preclinical multigenerational tests before the starting of large clinical trials.

### **Takeaway & research priorities**

- Preclinical evidence indicates that nano-antioxidants could be more effective than free antioxidants through: (a) targeting oxidative stress in the ovaries and adipose tissue, (b) downregulating the inflammatory pathway, and (c) having a positive effect on metabolic and reproductive parameters in the animal PCOS models. Nevertheless, the strong clinical evidence for the use of nano-therapy in the treatment of PCOS is still on its way. The following actions are considered the highest priority: development of a standardized GMP nanoformulation, determining the safe dosage, conducting reproductive toxicology studies, and carrying out randomized controlled trials to evaluate clinically significant outcomes (ovulation, pregnancy/live birth, insulin sensitivity, long-term cardiometabolic outcomes). (33)

## 5. IMMUNO-MODULATORS IN PCOS MANAGEMENT

PCOS has been accepted as having a large immune inflammatory component, thus immuno-modulatory strategies which are aimed at re-establishing immune homeostasis, diminishing pathogenic inflammation, and eventually enhancing the reproductive and metabolic outcomes have been chosen. PCOS immuno-modulators have a broad range of interventions starting from small-molecule drugs and nutraceuticals, and extending to biologics and microbiome-targeted therapies which reduce the pro-inflammatory signaling, change the immune cell phenotypes, or improve the regulatory mechanisms within the ovarian and systemic milieu. (34,35,36)

### **Natural vs. Synthetic Immuno-modulators**

Immuno-modulators constitute a very promising therapeutical approach in the PCOS treatment, especially that of the inflammatory and immune dysregulation issues in the foundation of the disease. Natural immuno-modulators come from plants, the ocean, or microorganisms and are curcumin, resveratrol, quercetin, epigallocatechin gallate (EGCG), and omega-3 fatty acids, for example. These compounds have multiple widespread actions such as that of inhibiting pro-inflammatory cytokines release activity, strengthening body's defence against oxidative stress, and modifying immune cells' activity, and, with their extra merit of being very safe and causing almost no toxicity in the long run, are the best candidates. On the other hand, synthetic immuno-modulators include pharmaceutical agents like glucocorticoids, biologics (e.g., TNF- $\alpha$  inhibitors, IL-6 blockers), and small-molecule drugs targeting intracellular signaling pathways such as NF- $\kappa$ B or JAK-STAT. Although synthetic agents may provide strong and precise effects, the trade-off is often linked with a higher risk of adverse drug reactions, immunosuppression, and financial barriers. The decision between natural and synthetic routes will be determined by the degree of immune dysregulation, and patient-related specification factors.

### **Mechanisms of Immune Modulation**

The immunomodulatory approaches for PCOS strive to bring back the immune equilibrium by adjusting the pro- and anti-inflammatory pathways. These agents, in terms of their action, might suppress the excessive production of pro-inflammatory cytokines like TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 thus leading to the reduced chronic low-grade inflammation that is there and contributes to insulin resistance and hyperandrogenism. One more important mechanism is the change in the T helper (Th) cell population, especially the decrease of Th1 and Th17 cell activities along with the increase of Treg cells that are responsible for the immune suppression which goes on in ovarian tissues where autoimmune-like processes occur. Macrophage polarization has also been enhanced with some immuno-modulators, leading to the anti-inflammatory M2 phenotype, which results in less tissue damage and more follicular repair. Furthermore,

nano-formulated immuno-modulators may assist in the precise delivery of the drug to the ovarian tissue, thus, making it more effective while at the same time lessening the exposure to the whole body system. The combination of antioxidant activity with immune regulation by these agents is the way they work in tackling the oxidative and inflammatory drivers of PCOS hence, they are eventually very beneficial to the ovulatory function and metabolic outcomes. (39,40,41)

## **6. NANO-FORMULATIONS FOR TARGETED DELIVERY**

Nano-formulation is a way where the carriers are of nanoscale and it is done mainly for therapeutic agents with better accuracy, stability, and effectiveness. In the case of PCOS treatment, these kinds of systems get the medicine to the right place in the body and that enhances the drug bioavailability, decreases the side effects, and makes the release of the drug controlled thus providing the patient with the right therapeutic action for a longer time. (42,43)

### **Nanocarriers and Drug Delivery Systems**

Actually, nanotechnology is a strong method for the delivery of drugs in Polycystic Ovary Syndrome (PCOS) treatment enhancement and gives the advantage of targeting the ovarian tissue and the metabolic organs connected to it precisely. Among the nanocarriers are liposomes, polymeric nanoparticles, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), dendrimers, and metallic nanoparticles (e.g., gold, silver, selenium), which ensure the stability of drugs, develop and release the drug into the body at a controlled rate, and prevent the drug from being degraded during the process. liposomes, have the phospholipid bilayer that helps the lipophilic and hydrophilic drugs enter and exit the cell, thus guaranteeing that the drugs are biocompatible and that systemic toxicity is minimized. Polymeric nanoparticles (e.g., PLGA, chitosan-based) allow for tunable drug release and surface modification for active targeting to specific receptors expressed in ovarian tissue. Metallic nanoparticles, when functionalized with ligands or antibodies, can be used not only for the delivery of drugs but also for theranostic applications—curative and contrast imaging at the same time. These nanocarrier-based systems can deliver a variety of therapeutic agents such as antioxidants, hormonal regulators, insulin sensitizers, and immuno-modulators that enable the multi-target action in PCOS pathophysiology. (44,45)

### **Enhancing Bioavailability and Target Specificity**

The poor bioavailability of many bioactive compounds, especially those that are plant-derived like polyphenols, peptides, and also hydrophobic drugs, is one of the main problems in the therapeutic treatment of PCOS. Nano-formulations, on the other hand, are able to deal with these limitations besides improving the solubility, protecting the drug from degradation via gastrointestinal or enzymatic means, and at last, aiding the drug to get across biological barriers via transcellular transport. Moreover, targeting ligands such as folic acid, transferrin, or antibodies used in the surface functionalization of nanoparticles, enhance the specificity of targets even more by selectively binding to the overexpressed receptors located on ovarian or endometrial cells. Furthermore, the mechanisms of controlled release in the nano-formulations keep the therapeutic concentrations for long periods of time so that the frequency of dosing is less and the patient compliance is improved. Moreover, these systems are able to reduce the off-target distribution and consequently the chances of systemic side effects which is already an important requirement for the long-term management of chronic diseases such as PCOS. Furthermore, advanced methods like the application of stimuli-responsive nanoparticles (which can be activated by pH, temperature or enzymatic activity) will be able to give the drug release right when it is needed in the diseased tissues, thus increasing the precision of the whole process. In general, the introduction of nanotechnology into the treatment of PCOS has turned the possibility of personalization, efficiency, and safety in treatment into reality, not to mention the fact that this could have a great impact on the outcome of the clinical practice. (46,47,48)

## **7. CHALLENGES, SAFETY, AND REGULATORY CONSIDERATIONS**

The integration of nano-based therapeutics into the management of PCOS represents a cutting-edge strategy, but its clinical application is constrained by several key challenges. While nanocarriers enhance drug solubility, bioavailability, and targeted delivery, they also introduce concerns regarding safety, toxicity, and long-term stability within the human body. Nanoparticles, due to their ultra-small size and high surface area-to-volume ratio, can interact with cellular components in unpredictable ways, potentially inducing oxidative stress, inflammatory responses, or genotoxic effects. Moreover, these technologies by minimizing the distribution of off-targets thereby reducing the chance of systemic side effects which is crucial in the long-term treatment of chronic disorders like PCOS. In addition, the use of advanced methods such as the application of pH-sensitive, temperature-sensitive or enzymatic activity-sensitive nanoparticles that can be activated provide the release of the drug in the target diseased tissues, hence, therapeutic precision is being further refined. Overall, the integration of nanotechnology with the treatment of PCOS has redefined the whole era of personalizing, effective, and safer treatment options that could dramatically alter clinical results. (46-48).

## Toxicity and Biocompatibility

The safety profile of nanoformulations is considered to be one of the most critical factors for their acceptance in clinical application for PCOS therapy. Nanoparticles can be made of different materials (like metallic, polymeric, lipid-based, or carbon-based) and thus can have different levels of toxicity and immunogenicity. Sometimes the presence of nanoparticles in the body for a long time can especially happen in vital organs like the liver, spleen, or ovaries and then lead to bioaccumulation and possibly organ dysfunction. The factors that affect toxicity include the size, shape, and surface charge of the particles as well as the type and extent of surface functionalization. For example, cationic nanoparticles have a higher degree of cellular uptake, however, they can also cause damage to cell membranes and lead to apoptosis. In addition, the breakdown products of some nanomaterials, particularly those that are non-biodegradable, can accumulate in the body and disrupt normal cellular functions. This is why a lot of in vitro and in vivo toxicity tests need to be done to provide good biocompatibility, with the possibility of oxidative stress induction, DNA damage, inflammatory cytokines release, and reproductive toxicity as part of the assessments. One way to deal with such adverse effects is to use non-toxic and super-disguised nanomaterials like PEGylated lipids or naturally obtained polymers. (52,53)

## Clinical Translation and Future Directions

The transition from laboratory innovation to clinical use of nano-assisted PCOS treatments is not easy and requires to overcome scientific, manufacturing, and regulatory logging. Even though preclinical results were very positive, most nano formulations still do not reach the clinic because of the lack of safety data, poor scalability, or non-reproducibility in manufacturing. The FDA and EMA are among the regulatory agencies that require detailed characterization of the nanoparticle physicochemical properties, the stability under physiological conditions, and the long-term safety profile to be established before their approval. Ethical dilemmas are also highlighted in fertility-related health concerns due to the direct influence on your reproductive potential and possible future generations having been affected. Research in the future should concentrate on creating multifunctional nanoplatfroms that could do drug delivery, antioxidant action, and immune modulation at the same time, and making use of real-time imaging for treatment monitoring as well. The combination of AI and machine learning might optimize the design of nanoparticles as well as their patient-specific response prediction. Therefore, personalized nano-therapeutics for PCOS could come closer to reality. Nanotechnology, together with physicians and regulators, will be needed in these collaborative efforts to bring about the visions of innovative, safe, efficacious, and accessible clinical solutions. (54,55)

## CONCLUSION AND FUTURE PROSPECTS

PCOS is a complex condition related to the endocrine and metabolic systems and the main inflaming, oxidative, and immunological imbalance processes are involved in the disorder's development and progression. The joining of these new highly effective therapeutic approaches such as nano-antioxidants, immuno-modulators, and targeted nano-formulations will probably lead to the possibility of eliminating the disease-causing mechanisms rather than merely easing the symptoms. The use of nano-based delivery systems has its own set of distinctive advantages, such as, enhanced bioavailability, selective drug delivery to the exact location, and lesser systemic side effects, all of which are plus points for the use of such systems in the long-term management of PCOS. On the other hand, one must first resolve issues like nanotoxicity, lack of compatibility with the body, regulatory blockages, and different patient responses before these systems can be adopted for clinical use on a large scale. The next step in the research should be to work on the production of multifunctional nanotherapeutics that have the properties of being anti-inflammatory, antioxidant and hormone-regulating all in one platform. In addition to that, the application of personalized medicine based on genetic, metabolic and immunological profiling might lead to the development of customized therapies whereby the results of the treatment would be improved. Also, strong preclinical studies and large-scale and well-controlled clinical trials should be conducted in order to show the drug's efficacy, safety and tolerability over the long term. The next decade might not only see but also experience such a shift in the management of PCOS, that is, from mere symptomatic relief to disease modification and prevention through targeted intervention, due to the continuous breakthroughs in the fields of nanomedicine, biotechnology, and immunopharmacology.

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