Endometriosis and PCOS: Two major pathologies linked to oxidative stress in women

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Introduction

Oxidative stress (OS) ensues when the detrimental activity of reactive oxygen species (ROS) prevails over that of anti-oxidants causing lipid peroxidation, protein carbonylation, and DNA damage and/or cell apoptosis. Moreover, reactive nitrogen species (RNS), such as nitrogen oxide (NO) with an unpaired electron, is also highly reactive and toxic (Agarwal et al. 2012, Doshi et al. 2012). OS has been known to participate in the pathogenesis of PCOS and endometriosis. Several OS biomarkers have been scrutinized by investigators, in the past, including MDA (malondialdehyde), protein carbonyl, TAC (total antioxidant capacity), SOD (superoxide dismutase), GPx (glutathione peroxidase), and GSH (reduced glutathione) to determine the role of OS in PCOS (Azziz et al.) and endometriosis (Jackson et al. 2005, Murri et al. 2013). Free radicals are known to impact several microenvironments in different biological windows, such as in the follicular microenvironment (Gonzalez et al. 2006, Murri et al. 2013). Both PCOS and endometriosis are associated with poor oocyte quality and infertility (Gupta et al. 2008, Goud et al. 2014, Huang et al. 2015). Our current review addresses the role of OS in both these disease conditions and the role of antioxidants and lifestyle modifications in preempting the impact of free radicals in PCOS and endometriosis.

Polycystic ovary syndrome (PCOS) is a multicomponent disorder affecting many adolescent girls as well as women of reproductive age, characteristically

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due to underlying hyperandrogenemia and hyperinsulinemia (Azziz et al. 2004). Obesity and enigmatic genetic factors are important underlying factors in PCOS. 50% of women diagnosed with PCOS have a high BMI characterizing them as obese and insulin resistance is seen in 50-60% of women with PCOS. The complex nature of PCOS is reflected on wide range of clinical presentation of affected females, which include but are not limited to both metabolic and reproductive disorders (Dabadghao et al. 2007, Azziz et al. 2009). Consequently, while European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) have agreed on a consensus definition of PCOS to help clinical investigators, the condition is recognized to have numerous clinical phenotypes (Rotterdam 2004, Rotterdam 2004). Endometriosis, on the other hand, is defined by the presence of functioning endometrial glands and stroma outside the uterine cavity (Kennedy et al. 2005). Endometriosis is an estrogen dependent disease affecting women in their reproductive years. (Sensky and Liu 1980, Meuleman et al. 2009).

Endometriosis: Pathogenesis and Clinical manifestations

Endometriosis is a devastating benign gynecologic disease, defined by the presence of functioning endometrial glands and stroma outside the uterine cavity (Kennedy et al. 2005). It is an estrogen dependent pelvic disease affecting women of reproductive age. The prevalence of endometriosis in the general population is as high as 10% while in women with pelvic pain, infertility, or both, the higher frequency of 35–50% is reported (Sensky and Liu 1980, Meuleman et al. 2009).

The major consequences of endometriosis are chronic pain and infertility. The main symptoms of the disease include repeated pelvic pain that peaks just before the beginning of menses, and then gradually lessens at the onset of flow. Other common symptoms include dysmenorrhea and dyspareunia. Nevertheless, some patients with endometriosis are asymptomatic and consequently, often remain undiagnosed (Abbas et al. 2012) until fertility is desired. The gold standard for diagnosing endometriosis is through laparoscopic surgery. Typical appearances of "gun powder like" lesions

in the pelvis followed by histopathological confirmation of endometriotic lesions are diagnostic of endometriosis (2010).

Pathogenesis and Clinical manifestations

The estrogen dependency of endometriosis is reflected by several acknowledged risk factors of the disease including early menarche, short menstrual cycles, menorrhagia, nulliparity, low body weight and obesity (Missmer and Cramer 2003, Matalliotakis et al. 2008). Genetic and epigenetic alterations as well as environmental pollutants, immunological dysregulation and a persistent inflammatory status also impose a risk of disease (Giudice and Kao 2004). Decreased estrogen levels during a state of ovulation suppression lessen the risk of endometriosis and the severity of its symptoms. Therefore parity, oral contraceptive use, and prolonged breast feeding are recognized as protective factors (Vercellini et al. 2011). Despite having being first described in the 19th century (1899), the exact origin of endometriosis is yet unknown and its etiology is still debated until today. However, there are multiple existing theories that try to illuminate the causes and enigmatic pathophysiology of the disease.

Sampson's implantation theory

The most generally accepted theory that explains the origin of endometriosis is the Sampson's retrograde menstruation and implantation theory (Sampson 1927). It proposes that endometrial cells are conveyed back through the fallopian tubes through retrograde menstruation. The location of many of the endometriotic lesions, such as the uterine ligaments, the rectosigmoid, and the cecum and ileocecal junction, the superior portion of the sigmoid mesocolon, and the right paracolic gutter and the pouch of Douglas, supports the theory (Chapron et al. 2006). The shared common denominator for all those regions is their increased contact with peritoneal fluid (PF), along with repetitive fluid flow (Chapron et al. 2006). Nevertheless, Sampson's theory cannot provide a passable explanation for all endometriosis characteristics. For instance, retrograde menstruation is present in 76 to 90% of women (Seli et al. 2003), while the implantation of the endometrial tissue and the further development of endometriosis occurs only in a portion of these women. Moreover, presence of endometrial lesions outside the abdominal cavity (Haga et al. 2014) or in the absence of a uterus (Troncon et al. 2014) pose a challenge to the theory.

Coelomic Metaplasia Theory

The Coelomic metaplasia theory proposed by Ferguson (Ferguson et al. 1969) suggests that the cause of endometriosis is metaplasia of the cells lining the pelvic endometrium (Metzger and Haney 1989). The theory proposes that endometriosis develops as a consequence of a number of stimuli induced by metaplasia, including endocrine and inflammation factors (Matsuura et al. 1999). The foundation of the theory lies in clinical evidence of endometriosis reported in subjects that depart from the typically-diagnosed population; as these also take in men, prepubertal girls, or women who never menstruated. Additional support to this theory can be found in the presence of endometriosis at unusual locations, even outside the abdominal cavity (Haga et al. 2014).

Embryonic Rest Theory

The embryonic rest theory proposes that rest cells of the Mullerian system may differentiate into endometrium under the stimulation of endocrine agents such as estrogen (Longo 1979). This theory may clarify reports of endometriosis observed outside the abdominal cavity or that of cases described in male patients treated with high doses of estrogens (Schrodt et al. 1980).

Vascular and Lymphatic Metastasis

The vascular and lymphatic metastasis theory advocates that endometriosis arises in the retroperitoneum and in sites not directly opposed to the peritoneum, and metastasize through hematogenous and lymphatic paths (Javert 1952). A well-known communication of lymphatics between the uterus, ovaries, tubes, pelvic and vaginal lymph nodes, kidney, and umbilicus exists allowing the spread of metastasis of endometrial cells (Javert 1952). The identification of endometrial cells in the veins of a uterus containing adenomyosis was previously reported by Sampson (Sampson 1927). Further support to the theory is that in an animal study, intravenous injection of endometrium was shown to cause pulmonary endometriotic lesion (Witz 1999). This theory can offer an explanation for infrequent cases of endometriosis occurring in locations remote to the peritoneal cavity.

Tissue injury and repair (TIAR)

The tissue injury and repair (TIAR) theory is a relatively recent one and claims that endometriosis is a consequence of trauma (Leyendecker

et al. 2009). The continuing uterine peristaltic activity prompts micro-traumatization and the activation of the mechanism of TIAR. The outcome of this is a local production of estrogen that meddles in the ovarian controlled uterine peristaltic activity, resulting in everlasting hyper-peristalsis and a self-continuation of the disease development (Leyendecker et al. 2009).

Composite Theory

The composite theory suggested by Javert et al. (Javert 1950) compile various processes, both direct and indirect, to address the pathophysiology of endometriosis. The theory is based on the assumption that endometriosis occurs due to a number of mechanisms: (I) endometrial tissue directly extended into the myometrium and to neighboring organs such as the bladder and intestine; retrograde menstruation through the tubes to the peritoneal cavity; (III) implantation of the endometrial cells in the peritoneal cavity; (IV) regional metastasis through the lymph nodes; and (V) distant metastasis via the hematogenic system.

The composite theory is striking, since it recognizes a multi-dimensional mechanism for the pathophysiology of endometriosis and therefore can support a reasonable explanation for the questions raised when inspecting each theory on its own (Witz 1999).

Polycystic Ovary Syndrome (PCOS): Definition

Polycystic ovarian syndrome (PCOS) is one of the most frequently seen endocrinopathies in adolescent girls and in women going through their reproductive years and exhibits a wide spectrum of clinical manifestations (Rosenfield 1997, Khan et al. 2006). Besides the features of hyperandrogenism, hyperinsulinemia and hirsutism commonly found in PCOS women, the primary cause of PCOS is probably multifactorial in origin (Norman et al. 2007) (Nardo et al. 2008). Hyperinsulinemia is associated with increased insulin resistance and it is a key feature of PCOS. Hyperinsulinemia is seen regardless of the body mass index value in some women with PCOS (Dunaif 1997). The resulting hyperinsulinemia together with central obesity, which is frequently encountered in PCOS patients, are components of the metabolic syndrome. One in five women with PCOS will also have metabolic syndrome, and its prevalence increases with age (Pasquali et al. 1999, Apridonidze et al. 2005, Cupisti et al. 2008). Metabolic syndrome is

an important factor for an increased predisposition in women with PCOS towards developing endothelial dysfunction, cardiovascular disease and type II diabetes. PCOS patients have been reported to have elevated markers of cardiovascular and endothelial disorders in addition to the familiar features of hirsutism, acne and anovulatory infertility (Fenkci et al. 2003, Kuscu and Var 2009). PCOS occurs among 4% to 12% of women within their reproductive years, and is the most frequent complex endocrine disorder among women (Khan et al. 2006). Even though older literature reports indicate 1 in 15 women are diagnosed with PCOS recent reports indicate a higher prevalence of PCOS in 12-21% of women (Apridonidze et al. 2005).

Diagnosis and Epidemiology of Polycystic Ovary Syndrome

The diagnostic norms for PCOS have been debated extensively at several forums and remain contentious due to heterotrophic phenotypic nature of the syndrome. PCOS is a complex genetic disorder and the genetic traits in combination with environmental as well as lifestyle factors lead to the development of this endocrinopathy (Conway et al. 1989, Azziz et al. 2009). Clinicians first noted the association between high androgen levels, hyperinsulinemia and Polycystic ovaries in 1935 and reported it as a composite syndrome: PCOS (Burghen et al. 1980). There is a gamut of clinical features that find expression in women with PCOS including impaired glucose tolerance (Legro et al. 1999), type II diabetes, elevated risk of hypertension and dyslipidemia, and elevated markers of endothelial dysfunction. These manifestations have further complicated the debate on defining PCOS (Franks 1989, Khan et al. 2006). The manifestation of clinical or biochemical hyperandrogenism and polycystic ovaries with regular cycles was broadly construed within the gamut of PCOS (Adams et al. 1986, Azziz 2003, Azziz et al. 2009). In earlier literature there were no well accepted norms for the diagnosis of PCOS. The NIH(National Institute of Health) was the first to introduce the criteria for PCOS diagnosis in 1990. The NIH launched diagnostic criteria characterizing PCOS as the combination of oligomenorrhea or amenorrhea and hyperandrogenemia, in the absence of non-classical adrenal hyperplasia, hyperprolactinemia and thyroid dysfunction (Rotterdam 2004) (Rotterdam 2004). Morphology of polycystic ovaries as assessed by ultrasound was not included in the criteria enlisted by the NIH. It was believed that wide ranging clinical

diagnostic criteria were required for clinicians to accurately diagnose the multi-etiological PCOS. However clinicians in Europe strongly believed that the ultrasound appearance of a polycystic ovary was a crucial diagnostic criterion to diagnose PCOS in women. A consensus document was produced as a result of the continued discussion, exchange of ideas between the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM). The consensus norms developed as a result of this exchange are commonly referred to as the Rotterdam 2003 criteria for defining PCOS. The new definition of PCOS recommends that the diagnosis of PCOS must be based on at least two of the three following criteria: (i) oligo- and/or anovulation; clinical and/or biochemical signs of hyperandrogenism and (iii) polycystic ovaries on ultrasonography and exclusion of related disorders (Rotterdam 2004, Rotterdam 2004). The ultrasound criteria for polycystic ovaries is defined as the presence of 12 or more follicles measuring 2 to 9 mm in diameter and /or an increased ovarian volume greater than 10 cm^3 on transvaginal ultrasound scanning. Presence of only one polycystic ovary is a sufficient benchmark for diagnosis of PCOS (Hart et al. 2004). The exceptions to these criteria are applicable to women who are taking oral contraceptives because of the modification induced in ovarian morphology (Hart et al. 2004). The NIH criteria (1990) considered total testosterone, free testosterone, androstenedione and DHEA as biochemical markers. In contrast the recent Rotterdam criteria (2003) deemed free androgen index, total testosterone and DHEA as diagnostic biochemical markers for PCOS. Effective screening for PCOS included eliciting family history of PCOS as per the Rotterdam 2003 criteria (Rotterdam 2004, Rotterdam 2004). The consensus document from ESHRE and ASRM introduced different clinical phenotypes: a) ovulatory women with polycystic ovaries and hyperandrogenism, and b) oligo-anovulatory women with polycystic ovaries, but without hyperandrogenism. The introduction of a diverse spectrum of clinical phenotypes in women with PCOS has introduced more debate on this heteromorphic disorder. (Norman 2001, Norman et al. 2007).

Pathogenesis of PCOS and Clinical Manifestations

Several studies have demonstrated a link between obesity, hyperinsulinemia and insulin resistance in women with PCOS, There are three main

contributory factors in the pathogenesis of PCOS and these are: 1) a continuum of insulin resistance, dysfunctional insulin regulation and hyperinsulinemia, 2) hyperandrogenemia and 3) genetic factors. The manifestations of PCOS resulting from the impact of these three underlying factors represent a wide range of symptoms and clinical features. The clinical features include hyperandrogenism, ovulatory disturbances and polycystic ovaries and metabolic syndrome. Metabolic syndrome results from hyperinsulinemia and associated obesity (Legro et al. 1999, Norman 2001, Apridonidze et al. 2005). Moreover, lifestyle factors such as inappropriate diet, lack of physical activity or stress can also initiate a series of changes and lead to the development of PCOS.

A - Insulin resistance and hyperinsulinemia

The most common clinical manifestation in PCOS patients is the presence of hyperinsulinemia and insulin resistance (Dunaif et al. 1989, Dunaif et al. 1990). The characteristic insulin resistance results in elevated insulin levels. There are two different clinical phenotypes in PCOS patients: obese with PCOS and lean patient with PCOS. There is an increased prevalence of glucose intolerance in PCOS and an oral glucose tolerance should be performed as a screening test in obese women with PCOS (Legro et al. 1999, Hart et al. 2004). The presence of hyperinsulinemia and glucose intolerance in PCOS patients has led to research at molecular level focused on insulin receptor and post-receptor defects (Diamanti-Kandarakis and Papavassiliou 2006). Hyperinsulinemia leads to overproduction of androgens by insulin action on theca cells. Dunaif et al. Literature reports state that elevated insulin levels lead to excessive serine phosphorylation, which inhibits the tyrosine kinase activity of insulin receptors, results in insulin resistance in PCOS patients (Dunaif et al. 1989). TNF alpha mediated insulin resistance in obese PCOS patients is also due to underlying dysinsulinemia and insulin receptor serine phosphorylation (Peraldi and Spiegelman 1998). The P450c17 enzyme activity leads to hyperandrogenism in PCOS women (Zhang et al. 1995). Hyperinsulinemia further enhances the effects of LH on theca interstitial cells leading to elevated androgen production as well as in halting the follicular maturation process (Franks 1989, Hillier and Tetsuka 1997, Nisenblat and Norman 2009).

B - Hyperandrogenemia

Elevated LH levels accompanied by LH insensitivity drive the

hyperandrogenism seen in patients with PCOS. Ovaries are the main source of elevated androgen levels (Rosenfield 1997). A disruption of the hypothalamic-pituitary gonadal axis and increased pulsatility of the gonadotropin releasing hormone center in the hypothalamus is the cause of the elevation of basal LH levels (Pastor et al. 1998). Progesterone hormone is incapable of down-regulating the GnRH neurons in the presence of hyperandrogenemia (Pastor et al. 1998). The increased pulsatility of the GnRH neurons triggers increased androgen secretion from the theca cells mediated by persistently high LH levels (Schuring et al. 2008). LH propelled hyperthecosis and increased androgen levels arrests follicular growth and impairs follicular maturation (Schuring et al. 2008).

Ovarian steroidogenic enzyme deficiencies of enzymes such as 3β-hydroxysteroid dehydrogenase type II and aromatase can trigger hyperandrogenemia and hypoestrogenemia resulting in PCOS-like conditions (Ehrmann et al. 1995). In addition follicles that are incapable of changing their internal milieu from androgen dominance to estrogen dominance will undergo a developmental arrest and manifest small arrested follicular cysts, a typical feature seen in PCOS. Hyperandrogenemia also is a consequence of malfunctioning adrenal steroidogenesis pathways contributing to a state of PCOS. The impact of the malfunctioning of adrenal steroidogenesis is a resultant decreased cortisol production, which triggers an overproduction of adrenocorticotrophic hormone (ACTH) in order to maintain normal serum cortisol level (Burghen et al. 1980, Khan et al. 2006). Increased ACTH production drives the adrenal androgen excess (Khan et al. 2006). The Hyperandrogenism in PCOS thus results from both ovarian and adrenal androgen hyper production, caused by similar underlying aberrant steriodogenesis pathways.

As stated above, compensatory hyperinsulinemia increases androgen production by the theca cells (Bremer and Miller 2008). Bilateral oophorectomy (Nagamani et al. 1986, Dunaif et al. 1990), or the administration of GnRH agonists to inhibit the increased GnRH pulsatility (Dunaif et al. 1990), or anti-androgenic compounds did not correct the hyperinsulinemia and insulin resistance in PCOS women. These studies provide evidence that the dysfunctional insulin actions precede the development of hyperandrogenemia in PCOS patients (Diamanti-Kandarakis et al. 1995).

C - Genetic Factors

Literature reports have highlighted that there is an increased risk of PCOS in women from affected families. (Sultan and Paris 2006). Metabolic perturbations start early in adolescence and also exist in adolescent relatives of women with PCOS, even before clinical signs of PCOS become evident (Carreau and Baillargeon 2015). A study done by Lin Li et al., among Chinese adolescents with PCOS reported some high risk factors associated with adolescent girls suffering with PCOS than in the control group. These risk factors included: early menarche (<12 years), family histories of menstrual disorders, diabetes, hypertension (Li et al. 2012).

Studies have reported a prevalence of 6-8% for PCOS in the general population (Kahsar-Miller et al. 2001), 35% of premenopausal mothers and 40% of sisters of PCOS women (who are not on hormonal therapy) (Franks et al. 1997, Kahsar-Miller et al. 2001) are likely to develop the disorder, suggests that the role of genetics in PCOS is one that is very likely. None of the genes has been shown to play an incontrovertible role in PCOS, This may be due to the limited selection of candidate genes. The heterogeneous nature of PCOS or lack of knowledge of disease pathophysiology, effect of environmental and lifestyle factors, such as diet and obesity, could modify gene expressions (Diamanti-Kandarakis and Piperi 2005, Escobar-Morreale et al. 2005, Goodarzi 2008). Investigators selected genes based on their hypothetical roles in PCOS by application of 100 candidate gene approaches and these were largely steered towards the following four select areas: (i) steroid biosynthesis and action; gonadotrophin synthesis and action; (iii) weight and energy regulation; and (iv)insulin secretion and action (Goodarzi 2008). The most investigated genes in PCOS are those related to steroidogenic abnormalities and insulin metabolism abnormality.

Genes involved in steroidogenesis such as cytochrome P450 17-hydroxylase/17,20-desmolase (CYP17) and aromatase gene (CYP19) have been probed to elicit intrinsic defects in metabolic pathways, which cause hyperandrogenemia in PCOS women. Further increased mRNA expression and enhanced promoter region of CYP17 genes of the theca cells has been reported in in young girls with PCOS compared with controls. Overexpression of 3 alpha-hydroxysteroid dehydrogenase and17-hydroxylase/17,20-lyase activities in PCOS women(Nelson et al. 2001) are reflected downstream as (Wickenheisser et al. 2000) elevated androgen

levels. Genetic mutation of the CYP19 results in loss of function of aromatase gene and leads to elevated androgen levels in PCOS women (Harada et al. 1992, Conte et al. 1994, Belgorosky et al. 2003).

Confounding results have been reported with investigation of the Insulin signal transduction pathway genes. Mixed results have been obtained when investigating variable number of tandem repeats (VNTR) polymorphism in the promoter region of insulin gene at 11p15.5. On the other hand Waterworth et al. (1997) report a robust association between class III VNTR of insulin gene allele and PCOS, Urbanek et al. (1999) did not find evidence to link class III allele and PCOS (Waterworth et al. 1997, Urbanek et al. 1999). Insulin receptor gene is another probable candidate gene since it seemed to be silenced in molecular studies (Diamanti-Kandarakis and Papavassiliou 2006). Insulin receptor function is disrupted when serine phosphorylation occurs instead of tyrosine phosphorylation in the insulin receptor(Dunaif 1997), which suggests that more studies are required on targets downstream of the insulin receptor gene(Diamanti-Kandarakis and Piperi 2005). However recent population based studies have been conducted in Han Chinese women and also European decent women (Welt and Duran 2014). These were genome wide association studies and have concluded that the 11 genetic loci are connected with PCOS (Welt and Duran 2014). These genome variants are inclusive of genes linked to gonadotropin action, genes related to type 2 diabetes and other genes which are not clearly associated with any pathologies.

Diagnostic Hormonal Markers in PCOS Patients

Hormonal markers are reported as an important methodology to evaluate aberrant steroidogenesis in PCOS. Several common markers that are examined in PCOS patients include LH, FSH, estrogen, sex hormone binding globulins (SHBG), insulin-like growth factor-1 (IGF-1), total/free testosterone, androstenedione, dehydroepiandrosterone (DHEA) and DHEA metabolite DHEAS, anti-Mullerian hormone (AMH), and 17-hydroxyprogesterone (Bremer and Miller 2008, Azziz et al. 2009).

Gonadotropin releasing hormone (GnRH) stimulates the release of LH and FSH from the pituitary gland. FSH controls the growth of the ovarian follicles, especially the granulosa cells. FSH acts on the granulosa cells of the

ovary and converts the androgens coming from the theca cells of the ovary to estradiol with the help of the enzyme aromatase. LH controls the theca cells of the ovary which are responsible for the production of androgens. High GnRH pulse frequency favors production of LH and low GnRH pulse frequency favors FSH production.(Blank et al. 2007). The hormone progesterone sends a feedback to the hypothalamus and subsequently slows the GnRH pulse frequency favoring FSH production initiating the next cycle. In PCOS, there is persistently high pulse frequency of GnRH resulting in elevated levels of LH, thereby elevated LH/FH ratio. This in turn stimulates ovarian androgen synthesis leading to hyperandrogenemia in individuals with PCOS(Bruni et al. 2010). The relatively low levels of FSH impair follicular development (Blank et al. 2007).

High insulin levels in PCOS not only stimulate ovarian androgen production, in women with PCOS but also directly down-regulate SHBG synthesis by the liver. Low SHBG levels are a good gauge of insulin resistance (Nestler et al. 1991). Androgen bioavailability in serum is restricted due to strong binding affinity of testosterone and dihydrotestosterone to SHBG (Nardo et al. 2008). Serum Bound testosterone is an erroneous reflection of androgen status in PCOS patients as there is reduced binding to SHBG. (Azziz et al. 2009). As a result, association constant for testosterone binding to SHBG and albumin are utilized to account for the metabolic changes by determination of the free androgen index (FAI = T/SHBG * 100%) (Vermeulen et al. 1999).

Another characteristic marker used is DHEA which is secreted from the adrenal zona reticularis. DHEA has several limitations such as low concentration and exhibits diurnal variation. DHEA has several limitations as a surrogate marker due to its diurnal variation and intra-subject variation and low serum concentration (Azziz 2003). The limitations of DHEA assessment can be overcome by measuring sulfate ester of DHEAS which is not subject to the variations and are a good indicator of adrenal androgen production (Lobo et al. 1981). It has been reported that 20-70% of women with PCOS have elevated DHEAS levels (Jaquish et al. 1996, Azziz et al. 2004, Kumar et al. 2005). There is a decrease in DHEAS levels with age (Kumar et al. 2005) and levels are regulated by the activity of DHEA sulfo-transferase (Hammer et al. 2005). Certain ethnicities such Mexican American group compared to the Caucasian American control group exhibit lower DHEAS levels (Kauffman et al. 2006). There are constraints

associated with assessment of DHEAS levels as barely 10% of women with high DHEAS levels will have hyperandrogenemia (Azziz et al. 2004). DHEAS measurement in PCOS women has to be construed with caution and needs to be combined with other hormonal markers for a complete picture (Azziz et al. 2009).

Literature reports have indicated that the AMH level is significantly higher in PCOS women (Cook et al. 2002) and this has been confirmed by increased production by granulosa cells cultured *in vitro* (Pellatt et al. 2007). Moreover, since AMH levels were positively correlated with antral follicle counts (Pigny et al. 2003), serum AMH measurements may serve as an additional diagnostic tool in patients below 35 years old (Norman et al. 2007). The role of AMH in modulating androgen levels is still contentious despite its well accepted role in follicullogensis. Pigny *et al.* (2003) reported an association between levels of AMH, testosterone and androstenedione only in PCOS women. Piltonen *et al.* (2005) reported an association between elevated AMH, testosterone and androstenedione in both PCOS women and control group (Pigny et al. 2003, Piltonen et al. 2005).

In conclusion, a combination of various hormones is utilized to provide a complete picture for the diagnoses of PCOS. Hormonal evaluation serves as supplementary diagnostic tools that need to be combined with the clinical scenario for diagnosis of PCOS by clinicians and scientists.

An Introduction to Oxidative Stress

Free radicals are characterized as being unstable and highly reactive. The free radicals acquire stability by stealing electrons from other molecules such as nucleic acids, proteins, lipids, carbohydrates. The reactivity of the free radicals, triggers cellular damage (Agarwal et al. 2005, Agarwal et al. 2008, Agarwal et al. 2012). The two major forms of free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS). Reactive oxygen species are typically formed by release of free electrons during oxygen reduction as a by-product of natural metabolic pathways (Inoue et al. 2003)). Most of mitochondrial generation of ROS occurs at complexes I (where NADH dehydrogenase acts) and III (where ubiquinol to ubi-semquinone to ubiquinone conversion occurs) of the electron transport chain (Inoue et al. 2003).

Free radical generation occurs during the processes of lipolysis and chemical generation and about 98% of inspired oxygen is reduced and the remaining 2% is incompletely reduced. The incomplete reduction of oxygen molecule results in generation of three major forms of ROS (Agarwal et al. 2005, Agarwal et al. 2008). The three main forms of reactive oxygen species are superoxide radical [O2-], hydrogen peroxide [H_2O_2], and hydroxyl [HO^-]. Electron leakage in the mitochondrial electron transport chain results in formation of the Superoxide radical. Molecular oxygen conversion to water gets preempted if it gains an additional electron during transit through complex IV in the electron transport chain (Agarwal et al. 2008). This process is a byproduct of ATP generation (Cadenas and Davies 2000). The free radical Hydrogen peroxide is formed from either dismutation of the superoxide radical or through oxidase enzymes. Hydroxyl ion gains three additional electrons and hence is most reactive of all the radicals. Cellular redox homeostasis is maintained by optimal antioxidant levels. Cellular damage results from an imbalance of free radicals and antioxidants (Inoue et al. 2003, Agarwal et al. 2008). Free radicals induce DNA damage by causing alterations in purine and pyrimidine bases. When the balance between antioxidants and oxidants tilts in favor of the oxidants, free radicals modify key transcription factors which can potentially alter gene expression.

A. Oxidative stress in endometriosis:

Oxidative stress (OS) is a result of an imbalance between free radicals and antioxidant defense mechanisms. Normally, reactive oxygen species (ROS) is scavenged by antioxidants both enzymatic and non-enzymatic ones (Gupta et al. 2006). An alteration of the physiologic balance between ROS and antioxidants result in the formation of increased OS, leading to various pathological effects within the body. The ROS are characterized by an unpaired electron in their outer orbit and therefore are extremely interactive with a selection of tissues containing proteins, lipids and nucleic acids (Figure 1).

In fact, ROS, through their presence in the peritoneal and oviductal fluid play an important role in reproductive physiology including ovulation, fertilization, embryo development, and implantation (Agarwal et al. 2012). Nevertheless, when ROS levels are elevated, it is not only destructive to the reproductive system, but they also produce additional free radicals, thus constantly increasing the OS (Agarwal et al. 2012) (figure 2).

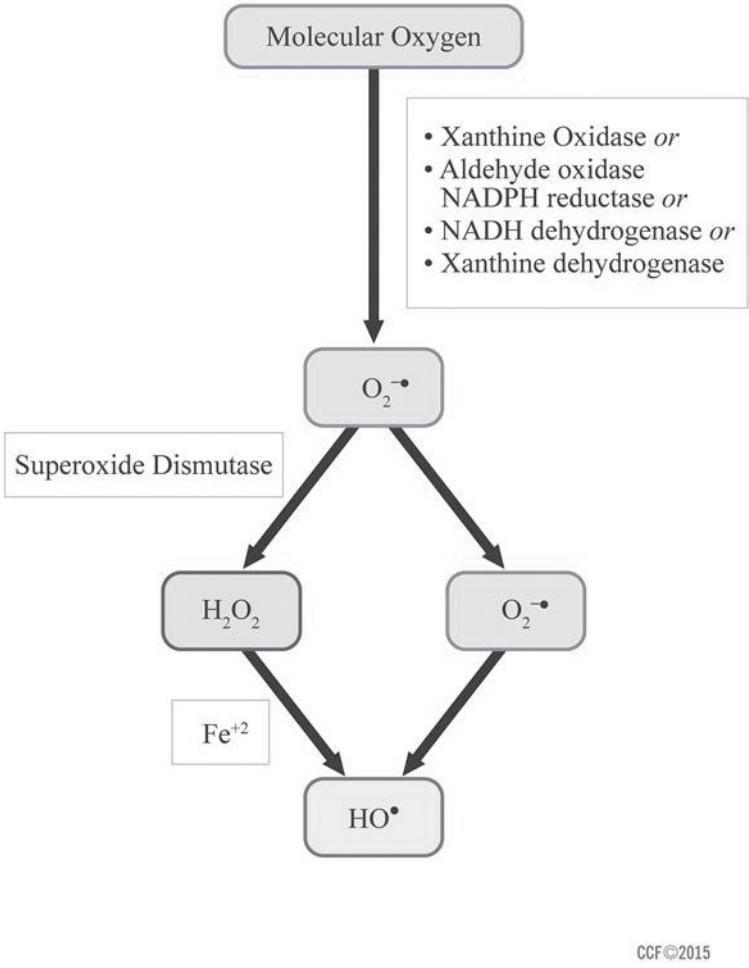

Figure 1: The redox pathway of superoxide formation.

Several studies described an increased level of OS markers in endometriosis patients in both the peritoneal and follicular fluid as well as in the endometriotic lesion (Szczepanska et al. 2003, Jackson et al. 2005, Polak and Kotarski 2010, Mier-Cabrera et al. 2011). Higher concentrations of malondialdehyde (MDA), pro-inflammatory cytokines (IL-6, TNF-alpha, and IL-beta), angiogenic factors (IL-8 and VEGF), monocyte chemo-attractant protein-1 and oxidized LDL (ox-LDL) have been identified in the peritoneal fluid of endometriosis patients (Mier-Cabrera et al. 2011, Polak et al. 2011,

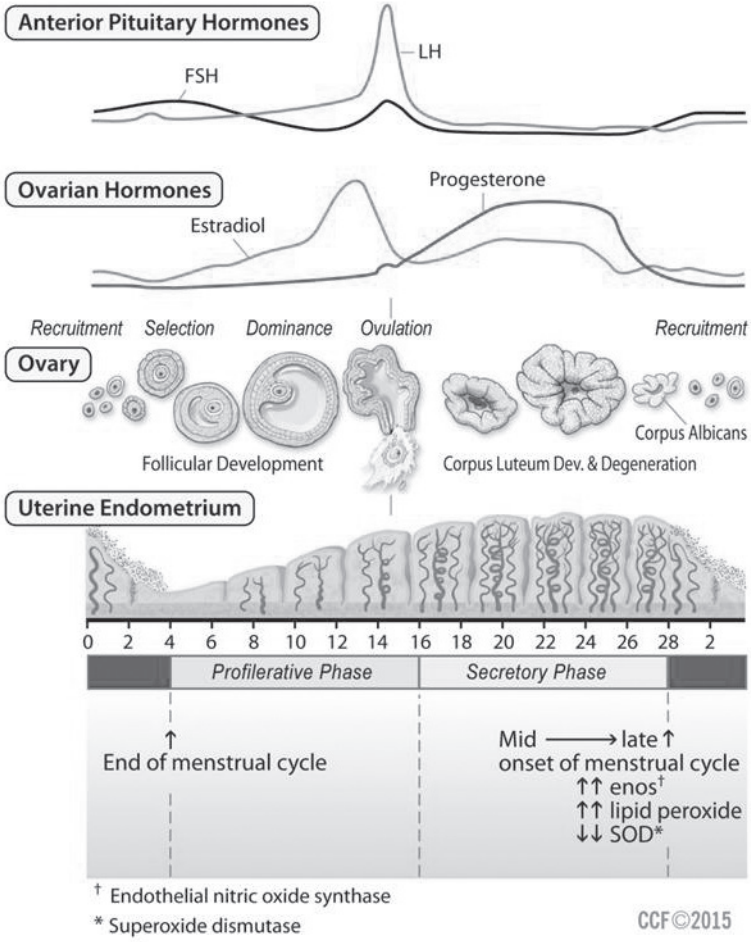

Figure 2: Redox balance throughout the endometrial and ovarian cycle.

Younis et al. 2014). All identified agents carry the ability to produce OS as pro-inflammatory and chemotactic cytokines. Moreover, recruitment and activation of phagocytic cells mediated through those cytokines are the main producers of both ROS and RNS (Gupta et al. 2006).

B - Possible ROS generators in endometriosis

Although the precise pathophysiology and etiology of endometriosis is not completely established, the chronic inflammation state that characterizes endometriosis is well established (Agic et al. 2006). The result of the

inflammatory process is an alteration in both the peritoneal fluid and the follicular fluid (Pellicer et al. 1998, Singh et al. 2013, Santulli et al. 2015) with an overproduction of an extensive variety of inflammatory mediators such as prostaglandins, metalloproteinases, cytokines and chemokines (Bulun 2009). In turn, the inflammatory process enables the adhesion, implantation and development of endometriotic tissue through an increase in ROS and at the same time intensifying further production of ROS and thereby increasing OS (Santulli et al. 2015).

The major role macrophages play in the production of ROS is well established (Alpay et al. 2006, Gupta et al. 2006). The increased amounts of activated macrophages in the PF of endometriosis patients is the main source of the large amounts of ROS observed in these patients (McLaren et al. 1996, Rakhila et al. 2014). The implanting endometrial tissue in peritoneal cavity is highly antigenic thus causing the activation of the peritoneal macrophages. Macrophage number and activity in the PF of endometriosis patients have been found to be increased, which results is an increased phagocytosis of the antigens as well as further accumulation of ROS. Indeed a possible association between the redox levels and the severity and progression of endometriosis has been suggested (Gupta et al. 2006).

Beside the previously described enhanced activity of macrophages, it has been found that the transcription factor NF-kB is significantly increased in endometriosis (Gonzalez-Ramos et al. 2007). It is mainly secreted by the activated macrophages present in the peritoneal cavity of women with endometriosis (Lousse et al. 2008). NF-kB is well known as a proinflammatory, mitogenic and antiapoptotic factor in many cell types (Van Langendonckt et al. 2004). Its increased activity causes further increases in the pro-inflammatory state of endometriosis. Being a transcription factor, it activates several genes and hence induces the progression of the disease (Gonzalez-Ramos et al. 2007). NF-kB binds to DNA and causes genes transcription in genes coding for cytokines, growth factors, angiogenic factors, adhesion molecules, and inducible enzymes such as nitric oxide synthase and cyclo-oxygenase (Viatour et al. 2005). Moreover, NF-kB activates adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) which allows the adhesion of endometrial cells prior to implantation in the peritoneal cavity. Figure 3 summarizes the major role NF-kB plays in the pathophysiology of endometriosis including ROS formation, increased transcription of genes

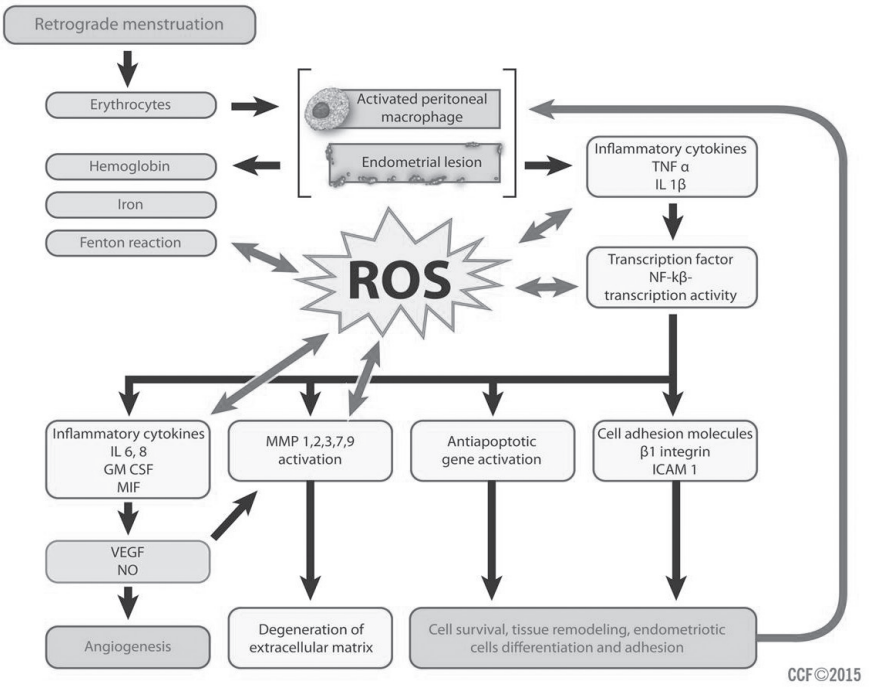

Figure 3: The vicious cyclical role of oxidative stress in the pathophysiology of endometriosis.

resulting in ectopic endometrium survival, adhesion and differentiation. Due to its major role in the development of endometriosis, NF-kB has been suggested to play a future part in the evaluation and surveillance of the endometriosis patient and may even be aimed as the treatment goal in endometriosis (Gonzalez-Ramos et al. 2007, Gonzalez-Ramos et al. 2008).

An additional source of OS formation is the matrix metalloproteinases (MMPs). The MMPs are a group of enzymes produced by endometrial stromal cells and some of them play a role in the normal physiological menstrual cycle (Koks et al. 2000). The regulation of MMPs in the normal endometrium is multifarious and is influenced by steroidal hormones, growth factors and cytokines (Kokorine et al. 1996). *In vitro* studies showed that exposure to progesterone suppresses the endometrial MMP protein and mRNA expression In endometriosis, the degradation of the extracellular matrix components is crucial for the ectopic endometrial tissue to adhere and invade. This degradation takes place due to the MMPs. In one study,

an abnormal expression of MMPs 2 and 9 were measured in women with endometriosis undergoing in-vitro fertilization reflecting poor oocyte and embryo development, and MMPs were suppressed by progesterone treatment (Singh et al. 2013). However, it is important to remember that above and beyond the capability of the ectopic endometrium to implant through the overactivity of the MMPs, that the same activity further generates ROS and thus increases OS (figure 3).

Another important component of endometriosis pathophysiology is the ability of the ectopic endometrial cells to establish blood supply in order to allow implantation and survival of the cells, much like cancerous cells. This ability is achieved through the angiogenic growth factor known as vascular endothelial growth factor (VEGF). In endometriosis, the inflammatory process activates the macrophages and other immune cells, which in turn produce high amounts of VEGF (Goncalves et al. 2015). Elevated amounts of VEGF in the peritoneal fluid of women with endometriosis as compared to healthy women have been reported (Kianpour et al. 2013). As angiogenesis is a necessity for the implantation and survival of cells, anti-VEGF treatment has recently been proposed as a possible treatment goal in endometriosis and has met with encouraging results (Song et al. 2014).

C - Markers of OS in endometriosis

Increased OS markers such as malondialdehyde (MDA), 8-isoprostane, lysophosphatidylcholine and 25-hydroxycholesterol were reported in endometriosis patients (Murphy et al. 1998, Szczepanska et al. 2003, Mier-Cabrera et al. 2011 and Polak and Kotarski 2010). These markers observed both in the PF and the follicular fluid of endometriosis patients are the products of the increased lipid peroxidation.

Lambrinoudaki et al. measured 4 major OS markers in histologically proven endometriosis patients as compared to healthy women (Lambrinoudaki et al. 2009). The following markers were evaluated: 1) Heat shock proteins (HSPs) these are intracellular proteins produced in order to provide cell protection against the consequences of infection or inflammation generated OS. The major representative of HSPs is heat shock protein 70; 2) Heat shock protein 70B' is a novel stress inducible gene of the HSP family; 3) Thioredoxin (TRX) which is a thiol oxidoreductase which regulates the cellular redox status and is released from cells in cases of elevated

OS. TRX protects the cell both directly through its antioxidant effects and indirectly via protein-protein interaction with signaling molecules; 4) Ischemia-modified albumin (IMA) an OS marker related to ischemia-reperfusion that was reported to be increased in several other elevated OS conditions as systemic sclerosis (Borderie et al. 2004) and ischemic stroke (Senes et al. 2007). The authors reported increased serum HSP 70b0 level in patients with endometriosis compared with controls regardless of the disease's stage. No significant difference in heat shock protein 70, IMA, and TRX levels were observed between patients with endometriosis and controls (Lambrinoudaki et al. 2009).

Despite the identification of OS markers elevation in endometriosis patients, the clinical implication of these markers is not well established yet, mainly due to lack of data. One of the recent studies addressing the clinical implication of the elevated OS markers in endometriosis patients was conducted by Carvalho et al. (Carvalho et al. 2013). While trying to predict endometriosis progression through OS markers, 6 different biomarkers of OS were assessed including 8-hydroxy-2-deoxyguanosine (8-OHdG), 8-oxoguanine DNA glycosylase (OGG1), protein carbonyl (PC), lipid peroxidation (LPO), reactive oxygen species (ROS), and total antioxidant capacity (TAC). These markers target protein, lipid, and DNA alternations. The aim of the evaluation was to quantify both endometriosis severity and progression and thereby to form a diagnostic marker for the 2 conditions. The authors reported significantly higher levels of 8-OHdG and PC as well as lower levels OGG1of in patients with endometriosis compared to controls. ROS, TAC, and LPO did not differ in all endometriosis stages and the control group. Using multivariable analyses a highly discriminatory predictive model was built with an ability to predict and distinguish between patients without endometriosis, stage I/II endometriosis, and stage III/IV. The authors concluded that OS as a biomarker of cell injury can be used as a trustworthy quantitative assay for the severity of endometriosis.

D - Possible ROS generators and markers of OS in PCOS

Oxidative stress results from an imbalance in the levels of free radicals and antioxidants and there is a disruption of the cellular homoeostasis which is favored by higher levels of oxidants. Oxidative stress generation impacts several processes in women with PCOS. A strong correlation has

been reported between oxidative stress and several clinical manifestations of PCOS such as central adiposity, insulin resistance, hyperandrogenemia, endothelial dysfunction, obesity, and infertility. Obesity, hyperinsulinemia and central adiposity associated with PCOS enhance the oxidative stress levels in women with PCOS (Olusi 2002, Keaney et al. 2003, Karadeniz et al. 2008, Verit and Erel 2008). Obesity, hyperglycemia and hyperinsulinemia are all known to be causative factors for ROS generation in PCOS (Kuscu and Var 2009, Kurdoglu et al. 2012, Nasiri et al. 2015, Sabuncu et al. 2001). The three factors can cause oxidative stress independent of each other in women with PCOS. Serum levels of oxidants and their end products such as MDA, Protein carbonyl, reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase, Paroxanase and glutathione transferase have been investigated to assess oxidative stress levels in PCOS (Verit and Erel 2008, Younis et al. 2014). On the other hand lifestyle modifications, weight reduction, optimizing caloric intake and diet loaded with antioxidants will favorably modulate the redox imbalance with a resultant decline in OS.

MDA as an OS marker in PCOS

Extraction of a hydrogen atom from a methylene group of the fatty acid chain initiates the peroxidation of unsaturated fatty acids. A molecular rearrangement leading to conjugated double bonds is needed to stabilize the carbon radical formed as a result of this reaction. Reaction with an oxygen molecule results in the generation of a reactive peroxyl radical which can abstract a hydrogen atom from lipids (Abuja and Albertini 2001).

Malondialdehyde (MDA) is one of the products of the decomposition of polyunsaturated fatty acids, and it is one of the stable end products of lipid peroxidation (Abuja and Albertini 2001, Kurdoglu et al. 2012). Several methods are available for quantification of lipid hydroperoxides and secondary lipid peroxidation products. MDA is most commonly measured by a thiobarbituric acid-reactive substances (TBARS) assay using a simple spectrophotometric method (Abuja and Albertini 2001, Bahmani et al. 2014) with a maximum absorption at 532-535 nm. While the assay for MDA is nonspecific, HPLC is a more accurate tool for MDA estimation.

Several Investigators have compared MDA levels in PCOS patients and age and BMI matched controls. The MDA levels have been investigated

in the blood, erythrocytes, and even in follicular fluid of PCOS patients. Blood MDA level, which was not specified whether it was measured from serum or erythrocyte in the literature, was found to be significantly higher in PCOS group as reported by Kuscu et al (Kuscu and Var 2009). The results showed that MDA levels are significantly higher in young, non-obese PCOS patients even in the absence of IR when compared with controls (Kuscu and Var 2009).

Zhang *et al.* (2008) demonstrated that the serum MDA levels in PCOS patients was significantly higher than that of the control group. A negative point of this study was that some of the important patient characteristics, such as BMI and age, were not elicited (Zhang et al. 2008). Several other literature reports have also demonstrated no significant differences in the MDA levels of PCOS patients versus age and BMI matched controls (Dursun et al. 2006, Karadeniz et al. 2008). Furthermore, MDA levels were found to be similar in the PCOS patient group, whose homeostasis model assessment-estimated insulin resistance (HOMA-IR) index was below and above the cutoff value, indicating that Insulin resistance did not exert any impact on MDA levels in women with PCOS.

Markers of Protein Oxidation in PCOS

Protein oxidation is an end product of oxidative stress which results from damage to the protein molecules induced by free radicals. Colorimetric assay is utilized to measure the protein cabonylation. Protein carbonylation(PC) is measured by the reaction of the serum with the compound dinitrophenylhydrazine. Fenkci *et al.* (2007) demonstrated higher protein cabonylation levels in lean women with PCOS. The protein carbonylation levels showed a correlation with the fasting insulin in PCOS patients (Fenkci et al. 2003) and other nvestigators have shown elevated PC levels independent of insulin levels(Kurdoglu et al. 2012). In a study by Piombini et al., follicular protein oxidative profile was investigated and correlated with oocyte quality in women with PCOS undergoing IVF (Piomboni et al. 2014). The assessment was done by a reduction in the free thiol (-SH) groups which are present as cysteine residues. Free thiol groups were labeled and this was followed by a two dimensional gel electrophoresis (Piomboni et al. 2014). Treatment with metformin and D-chiro-inositol (DCI) resulted in a significant decrease in the free-SH groups demonstrating the antioxidant actions of both metformin and DCI.

Protein biomarkers and linkage with OS in PCOS

Recent proteomics studies are seeking to investigate protein biomarkers and their association with inflammation and OS in PCOS patients. The selection criteria applied to the PCOS cases and control cases are stringent and potentially contribute to the quality of the studies. Koninger et. al, investigated Afamin protein: a binding protein for the antioxidant vitamin E. Significantly higher serum levels of afamin were detected in PCOS patients in contrast with the controls (Koninger et al. 2014)). A significant correlation was also reported between afamin levels and BMI, fasting glucose, HOMA-IR and a significant negative inverse correlation with SHBG levels. However on a multivariate regression analysis conducted by the investigators HOMA-IR remained to correlate with afamin levels (Koninger et al. 2014). Elevated serum afamin levels were believed to be a consequence of IR in the PCOS patients. Hence the authors propose that in PCOS patients with more marked IR and hyperandrogenism there will be even greater rise in afamin concentrations. Afamin is reported to be a better predictor of glucose intolerance developing in PCOS patients.

Significant expression of Peroxiredoxin (Prx) and glutathione S-transferase M3 (GSTM3) which are both antioxidant proteins have been reported in adipose tissue of PCOS patients. In order to offset the higher ROS levels in PCOS the study has reported that the Prx levels decrease and a compensatory elevation of GSTM3 levels was reported (Corton et al. 2008).

Oxidative stress indicators such as transferrin have been measured in follicular fluid of PCOS patients. Iron is a redox inducing molecule and is neutralized when transferrin binds the iron molecule. Dai and Liu have reported elevation in follicular fluid transferrin levels in women with PCOS undergoing controlled ovarian stimulation (Dai and Lu 2012). Elevated expression of the matrix metalloproteinase Prolidase was demonstrated in serum of PCOS patients (Hilali et al. 2013)). Prolidase is a protein that causes remodeling of extracellular matrix and is associated with OS. Prolidase activity is increased also in patients with cardiovascular diseases and could emerge as a potential new biomarker for PCOS to be employed in combination with clinical manifestations to establish PCOS diagnosis. Validation studies are needed to identify a reliable protein biomarker in women with PCOS (Gupta et al. 2014).

A. Role of antioxidants in endometriosis

Antioxidants are natural defense mechanisms against OS. Antioxidant molecules act as scavengers by donating an electron to the ROS, thereby deactivating them. Furthermore, antioxidants can decrease ROS production or even repair OS-induced damage (Alpay et al. 2006). Antioxidants may be enzymatic like catalase and glutathione peroxidase, or they can be non-enzymatic as vitamins A, C, and E.

Decreased levels of anti-oxidants have been observed in both peritoneal and follicular fluid of women with endometriosis compared to non-endometriosis women (Liu et al. 2001, Shu et al. 2013, Huang et al. 2014). The well-established lower levels of antioxidants, such as superoxide dismutase and glutathione peroxidase, in the peritoneal fluid of women with endometriosis have strengthened the proposed major role of OS in endometriosis pathophysiology as well as in endometriosis-induced infertility (Szczepanska et al. 2003).

The revelation of the role of OS as a major component of endometriosis pathophysiology has led to ongoing research with OS reduction as the treatment goal of endometriosis. Since the identification of OS as part of the pathologic process of endometriosis is still relatively new, most of the published studies on newer and advanced antioxidants are either in vitro or animal studies.

Vitamins C and E are widely studied anti-oxidants. The intake of these vitamins can be either through diet or supplementation. Animal studies revealed that vitamin C supplementation can decrease the size of endometriotic lesions and decrease the content of natural killer cells, reflecting a reduction in the inflammatory activity in endometriosis and resulted in decreased OS (Durak et al. 2013). Moreover, an inverse association between dietary intake of vitamins C and E and endometriosis diagnosis has been reported (Darling et al. 2013). Vitamins C and E were also shown to effect peripheral OS markers of endometriosis patients. Mier-Cabrera et al. (Mier-Cabrera et al. 2011) performed a randomized, double-blind trial treating endometriosis patients with vitamins C and E or placebo. The results indicated that both MDA levels and lipid hydroperoxides significantly decreased after 4 months and 6 months respectively of treatment with Vitamins C and E supplementation as compared to the control group. Moreover in a double

blind clinical trial, vitamins C and E supplementation were reported to decrease chronic pelvic pain, dysmenorrhea and dyspareunia in women with pelvic pain and endometriosis (Santanam et al. 2013).

The identification of OS as a major component of the pathophysiology of endometriosis and the encouraging primary results of the antioxidant effects on the different aspects of the disease has evoked further investigation using newer antioxidants.

Resveratrol (trans-3,5,40-trihydoxystilbene) is a natural flavonoid found mainly in grapes and in red wine. Resveratrol is known for its strong antioxidant competence as well as antineoplastic and anti-inflammatory effects (Amaya et al. 2014). For those reasons, it is widely studied and used in other clinical fields such as cancer and diabetes research. Resveratrol can impact endometriosis development and progression through several pathways. It can reduce inflammatory response through reduction of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), IL-6, IL-8, it may inhibit angiogenesis through VEGF reduction and influence cell survival through nuclear factor κB (NFκB) inhibition (Csaki et al. 2009). Animal and in-vitro studies showed that applying high doses of resveratrol decreased human endometrium proliferation possibly through its impact on estrogen receptors (Amaya et al. 2014). Further animal studies reported that resveratrol reduced endometrial lesion size, cell proliferation, vascularization, inflammation and OS lipid peroxidation markers (Ergenoglu et al. 2013, Rudzitis-Auth et al. 2013, Ozcan Cenksoy et al. 2014, Yavuz et al. 2014, Bayoglu Tekin et al. 2015). Moreover, in a study of human subjects including patients, who failed to achieve symptomatic relief using the traditional combined oral contraceptive treatment, the addition of resveratrol led to a reduction in pain scores in 82% of patients after 2 months (Maia et al. 2012).

Similar encouraging results were reported on epigallocatechin-3-gallate (EGCG). EGCG is a polyphenol abundantly found in green tea. It is well-known for its anti-oxidative, anti-mitotic, and anti-angiogenic features (Nagle et al. 2006). EGCG was reported to modify a number of carcinogenic mechanisms including cell proliferation, invasion and apoptosis (Beltz et al. 2006). Laschke et al. (Laschke et al. 2008) studied the impact of EGCG on estrogen-induced activation of endometrial cells. The authors observed an inhibition of the estrogen-stimulated activation, proliferation and VEGF

expression of ectopic endometrial cells. In the same manner, angiogenesis was inhibited without affecting the development of blood vessel of the ovary. While comparing the impact of Vitamin E and EGCG on angiogenesis using an animal model, EGCG, but not Vitamin E inhibited angiogenesis of the ectopic endometrial tissue (Xu et al. 2011).

Another important factor which enables the development of endometriosis is fibrosis, which is a consequence of the increased inflammatory reaction and of OS as part of endometriosis pathophysiology. EGCG was reported not only to inhibit cell proliferation and invasion to the peritoneal tissue but also to reduce the mRNA expression of fibrosis markers in ectopic endometrial cells. Animal experiments showed that EGCG prevented the progression of fibrosis in endometriosis (Matsuzaki and Darcha 2014).

In a similar manner, other anti-oxidants as melatonin, xanthohumol and other were reported to have a desired inhibiting effect on endometriotic lesions implantation and survival (Paul et al. 2010, Rudzitis-Auth, 2012, Wang, 2013). All these results clearly indicate that although the pathophysiology of endometriosis is not clearly understood, OS is well identified as a major key player in the pathophysiologic process enabling the progression of the disease and in determining its severity. Future studies are required to validate the preliminary results and eventually aim at reducing as the treatment goal of endometriosis.

B - Role of Antioxidants in PCOS

Excess ROS is scavenged by Antioxidants in order to counter the potential for significant cell damage by elevated ROS. There are two major classes of antioxidants: enzymatic and non-enzymatic. Enzymatic antioxidants include superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px). Non-enzymatic antioxidants include glutathione (GSH), a-tocopherol (vitamin E), β-carotene, ascorbate (vitamin C), taurine, L-carnitine, coenzyme Q10, etc (Agarwal et al. 2005). There are three SOD isoforms in eukaryotes: manganese SOD (Inoue et al.), copper/zinc SOD (Cu/Zn-SOD), and extracellular SOD (EC-SOD). Cellular damage can result directly by the production of H_2O_2 within the cell and in addition by generation of $HO^{\cdot}$ ion which can also induce subsequent cellular damage. H_2O_2. Radical is neutralized by Catalase, a manganese or heme-containing enzyme which functions to rapidly dismutate H_2O_2 to water and oxygen (Agarwal et al.

2008). Catalase is mainly found in peroxisomes, perhaps because of the large number of $H_2O_2^-$-producing oxidases found in these organelles, while lower levels are also found in mitochondria and the cytosol (Agarwal et al. 2008).

Literature reports present the evidence for depletion of antioxidants leading to OS and PCOS. OS and antioxidants do exert an impact in causing PCOS with its varied clinical manifestations of metabolic syndrome including diabetes, obesity, coronary diseases and cardiovascular diseases. Further research should be conducted in revealing which patient populations will benefit more from supplementation of antioxidants.

C - Significance of TAC in PCOS

Free radicals are quenched by the serum antioxidants at the generalized and by native antioxidants at local level. Total antioxidant capacity (TAC) is the total quenching capacity of the antioxidants either determined at the systemic level or in localized biologic windows such as the follicular fluid. Various detection assays have been described, including the spectrophotometric assay in which long lived 2,2'-Azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) radical cation is measured. ABTS radical is formed by the incubation of ABTS with a peroxidase (metmyoglobin) and hydrogen peroxide. The principle of the assay is to measure the ability of aqueous and lipid antioxidants to inhibit the oxidation of ABTS to ABTS+ (Mahfouz et al. 2009, Gupta et al. 2011). Trolox standards are utilized for comparison to evaluate the capacity of the antioxidants to prevent ABTS oxidation, which is a brand name for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble derivative of vitamin E. All antioxidants such as vitamins, proteins, lipids, glutathione, uric acid, etc are quantified by the assay and it measures the composite antioxidant capacity of all these components (Mahfouz et al. 2009 and Gupta et al. 2011).

Significantly lower level of TAC was reported in PCOS patients compared with the age-, BMI-, and smoking status-matched controls by Fenkci et al. (2003 and Fenkci et al. 2003). The study showed an inequity in the oxidative status in women with PCOS women may contribute to the increased risk of cardiovascular diseases. Insulin resistance may have a detrimental effect as there was a negative correlation between IR and antioxidant levels.

In contrast to some of the studies, Verit *et al.* reported significantly higher

TAC levels in PCOS patients compared to age- and BMI-matched controls. The study by Verit et al established that higher levels of antioxidants in PCOS patients independent of obesity and insulin levels. The contradictory results reported in this study point towards detrimental effects of TAC in PCOS. Elevated total oxidative stress was proposed to lead to a compensatory increase in TAC to offset the high levels of free radicals (Verit and Erel 2008). The conflicting results can be explained by factors such as different patient selection criteria, presence or absence of complications of PCOS.

D - Role of antioxidant SOD as a marker in PCOS

SOD induces the conversion of superoxide to H_2O_2, a toxic substance that is converted to water by GSH-Px (Combelles et al. 2010). High SOD levels may preempt the free radicals and could have prevented endothelial dysfunction. An adequate antioxidant response against such an inherent oxidative load in PCOS patients may lead to prevention of endothelial dysfunction in these women. Kuşçu et al. (2009) demonstrated that SOD levels were significantly higher in PCOS group compared to control group (Kuscu and Var 2009). In this study the PCOS patients were further divide into two subgroups: IR- and IR+. It was demonstrated that SOD levels were significantly higher in both subgroups compared to the control. This elevation may have been due to defensive mechanisms of the body (Kuscu and Var 2009). The subjects enrolled in this study were relatively young (mean age 23.8±4.37) with greater ability to cope with higher levels of ROS production.

Zhang et al. (2008) demonstrated that the serum SOD level in PCOS patients was significantly lower than that in the control group (Zhang et al. 2008). However the study by Zhang et al did not enlist the patient inclusion criteria and hence was a weak study. In a recent study examined both systemic and follicular SOD levels in patients with PCOS undergoing IVF/ICSI. The investigators demonstrated significantly lower levels of SOD both in the serum and the follicular fluid of PCOS patient versus the control group (Seleem et al. 2014).Seleem et al concluded that the serum SOD levels maybe a good parameter to measure systemic OS in PCOS.

E - GPx: an antioxidant marker in PCOS

Glutathione peroxidase did not differ between PCOS group and healthy control group in a study by Sabuncu *et al.* (2001). An increased level of GPx is expected in PCOS due to increased production of the H_2O_2 free

radical (Sabuncu et al. 2001). Obesity per se may cause a depletion of the protective antioxidants such as SOD and GpX. Significantly lower levels of serum MDA and higher levels of erythrocyte SOD and GpX have been reported in PCOS subjects with normal BMI as well (Olusi 2002).

F - GSH antioxidant marker levels in PCOS

GSH is determined by adding 5,5'-Dithiobis(2-nitro-benzoic acid), which is a disulfide chromogen that is readily reduced by sulfhydryl compounds to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to the GSH concentration (Beutler et al. 2014).

GSH was significantly lower in the PCOS patient group than in the control group as demonstrated by Sabuncu *et al.* (2001) (Sabuncu et al. 2001). In its efforts to neutralize the ROS and other free radicals GSH gets depleted. GSH may have been partly related to insulin resistance. In accordance with the findings of Sabuncu *et al.* (2001), Dincer *et al.* (2005) also found the GSH levels to be significantly lower in women with PCOS than in the control group (Dinger et al. 2005).

Comprehensive assessment of the role of antioxidants, LSM in PCOS

Several antioxidants such as folate, L-carnitine and *D-chiro-inositol* have been investigated for their role in countering oxidative stress in PCOS patients and improving endocrine profiles and outcomes. Addition of antioxidant supplementation to ovulation induction or other treatment modalities is reported to improve outcomes in patients with PCOS (Seleem et al.). There is enhancement of ovulation induction rates and pregnancy rates in the supplemented group. Folate supplementation in a dosage of 5mg/day for a short period of 8 weeks in women with PCOS significantly reduced the inflammatory and oxidative stress markers and increased the antioxidant capacity (Bahmani et al. 2014). This study was conducted as a double blind placebo controlled trial and showed that there were beneficial effects of folate supplementation with significant reduction in the levels of 3 markers: plasma homocysteine, HOMA-B (homeostatic model assessment

of beta cell function), and plasma MDA (Bahmani et al. 2014).

Piombini et al investigated the OS levels in follicular fluid and the impact of antioxidant D chiro-inositol supplementation on OS levels. The antioxidant D-chiro-inositol supplementation was given 3 months prior to starting the ovarian stimulation protocol in a group of women with PCOS. Metformin was given in the same period to a second group of women with PCOS. Both Metformin and D-chiro-inositol resulted in significant recovery of free-SH groups (Piomboni et al. 2014). The beneficial impact was seen in the recovery of a higher number of competent MII oocytes.

In a large randomized controlled trial, woman with clomiphene citrate resistant-PCOS were selected and randomized to clomiphene citrate with placebo versus clomiphene citrate with L carnitine supplementation from day 3 through day 7 of their cycle. Supplementation with L-carnitine in patients resistant to clomiphene citrate significantly improved ovulation rates and successful pregnancy outcomes. L-carnitine acts by reducing the oxidative stress in the follicular microenvironment (Ismail et al. 2014).

Conclusion

Our chapter review highlights the large body of evidence that exists in the literature linking oxidative stress with two enigmatic pathologies: endometriosis and PCOS afflicting millions of women worldwide. Several oxidative stress markers have been studied in different biological windows in endometriosis and PCOS. Studies have demonstrated an imbalance in the levels of free radicals and antioxidants disturbing the cellular homeostasis and leading to reproductive and metabolic complications. Measurement of biomarkers of OS is also a known controversial issue. Standardized methodology and units of measurement should be used globally in assessment of the various markers across different laboratories.

There is escalating evidence in the current literature validating the etiological relationship between oxidative stress, metabolic syndrome, PCOS and its long term complications. Results of several studies in endometriosis clearly indicate that although the pathophysiology of endometriosis is not clearly understood, OS is well identified as a major key player in the pathophysiologic process enabling the progression of the disease and determining its severity. Future studies are required to validate the

preliminary results eventually aiming at OS reduction as one of the treatment goal of endometriosis and PCOS. Several researchers are investigating the impact of reducing visceral adiposity in PCOS patient and whether altering adiposity leads to reduced markers of oxidative stress, improved insulin resistance and the amelioration of the clinical symptoms of PCOS. Individualized and personalized treatment may need to be designed for each patient to ameliorate oxidative stress by lifestyle modifications as well as supplementation with appropriate antioxidants in either of the diseases. Lifestyle modifications entail weight reduction, exercise training and diet modification in order to modulate the OS levels. Treatment algorithms have been adopted where certain fertility treatments are not made available to obese patients because of poor treatment outcomes and will be made available only subsequent to adoption of LSM by these patients. Clinical trials need to be conducted as part of future research investigating the role of OS markers and antioxidants in diagnosis of the disease, as well as monitoring the severity of the condition, as a prognostic marker to evaluate therapeutic response and modifying outcomes with both diseases. Future studies are required to validate the preliminary results and eventually aim at reducing OS as the treatment goal of PCOS and endometriosis respectively.

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