

Article

Role of oxidative stress in endometriosis



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Abstract

Endometriosis is a chronic pleomorphic disorder with pelvic or systemic manifestations, and is characterized by the presence of endometrial glands and stroma. In the United States, the prevalence of the disease is estimated to range from 2 to 50% in women of reproductive age. No single theory can explain the histogenesis and the pleomorphic manifestations of endometriosis. Endometriosis is being reported in younger age women and manifesting with increasing severity, hence the need to understand the role of oxidative stress (OS) in endometriosis. The presence of elevated concentrations of free radicals and lowered antioxidant potential leads to OS. The development of OS in the local peritoneal environment may be one of the links in the chain of events leading to endometriosis. Redox levels may modulate the severity and the dynamics of endometriosis and progression of the disease. OS has been implicated in infertility associated with endometriosis. Recent literature reviewed investigates the role of molecular mechanisms and genetic pathways that may modulate cellular response to OS. Antioxidant supplementation, immunomodulators, and selective progesterone receptor modulators with antioxidant effects have been investigated as possible treatments for endometriosis, but compelling evidence on the benefits of the various modalities is lacking. Results of the limited number of animal and human trials need to be corroborated by larger randomized controlled trials.

Keywords: antioxidants, endometriosis, oxidative stress, progesterone receptor modulators, reactive oxygen species

Epidemiology of endometriosis

Endometriosis is a chronic pleomorphic disorder characterized by the presence of endometrial glands and stroma outside the endometrial cavity. Endometriosis is a common benign disease in which endometrial glands and stroma grow outside the uterus, generally in the peritoneal cavity, but also in the pleural cavity, liver, kidneys, gluteal muscles, and the bladder (Pritts and Taylor, 2003). The pathology of endometriosis is not easily tracked epidemiologically, due to the postulated large number of women in the general population living with the disease and not receiving treatment (Scarselli *et al.*, 2005). In the USA, the prevalence of the disease is estimated to range from 2 to 50% in women of reproductive age. Because infertile women are more prone to undergo laparoscopy (which is the gold standard in

the diagnosis of endometriosis) in order to identify the cause of their infertility, the prevalence of endometriosis in infertile women has been reported to range between 20 and 50% (Pritts and Taylor, 2003). The average age for endometriosis diagnosis is 28 years, although it has also been found in women aged 12–80 years (Pritts and Taylor, 2003).

Endometriosis is an oestrogen-dependent disease associated with pelvic pain and infertility (Scarselli *et al.*, 2005). Cyclic pelvic pain starting before the menstrual period and continuing throughout the cycle until the end of the menstrual flow is characteristic of endometriosis. Pain in the musculoskeletal regions is also common, although not universal (Pritts and Taylor, 2003). The mechanisms responsible for infertility in women with endometriosis are presently unknown, although

changes in the composition of peritoneal fluid (PF) may be an important factor (Szczepanska *et al.*, 2003). An increase in number of PF macrophages and cytokines produced by these macrophages has been reported (Szczepanska *et al.*, 2003).

Infertility associated with endometriosis may also be caused by mechanical blockage of the sperm-egg union by endometriomata and pelvic anatomy malformations (Pritts and Taylor, 2003). Additional systemic and peritoneal abnormalities have been well documented in endometriosis. Systemic immunological abnormalities include increased immunoglobulin production, increased presence of helper T cells, lack of lymphocyte-mediated cytotoxicity against the endometrium, defective natural killer cell activity, and a decrease in suppressor cell activity. Endometrial stromal cell proliferation, lymphocyte proliferation, decreased sperm binding to zona pellucida, increased cyclic activation of macrophages, and the presence of non-organ specific auto-antibodies are characterized as peritoneal immunological abnormalities (Gleicher and Pratt, 1993).

The pathogenesis of endometriosis has been much debated, and various hypotheses have been postulated, such as the implantation, coelomic metaplasia and lymphatic dissemination theories. Sampson's implantation theory proposed that intraperitoneal menstrual reflux leads to spillage of endometrial cells in the peritoneal cavity. The spillage results in implantation of the endometrial cells to the peritoneum and development of endometriotic lesions. Halban (1925) proposed that endometrial cells could disseminate through lymphatic and haematogenous pathways. The metastatic theory explains the causation of extraperitoneal and distant endometriotic lesions.

The formation of the typical 'chocolate cysts' was first proposed to be caused by the inversion and progressive invagination of the ovarian cortex. This hypothetically occurs after the accumulation of menstrual debris from an already bleeding endometriomata present on the ovarian surface (Vignali *et al.*, 2002). Another hypothesis consists of metaplasia of the coelomic epithelium. Since the ovary and Müllerian ducts are both derived from coelomic mesothelium, the Müllerian theory proposes that ovarian germinal epithelium attempts to recapitulate Müllerian duct-derived endometrium (Oral and Arici, 1997). In an extension of the coelomic metaplasia theory, the induction theory proposes that endogenous biochemical factors induce endometrial tissue development from undifferentiated cells (Oral and Arici, 1997). The most widely accepted theory, however, (which is also known as Sampson's theory and the implantation theory) suggests that reflux of viable endometrial tissue through the Fallopian tubes results in the formation of endometriotic lesions after tissue implantation on the peritoneal surface or pelvic organs (Vigano *et al.*, 2004). Lateral asymmetry of ovarian endometriotic cysts has also been noted. The cysts appear more frequently on the left side of the cavity, due to the presence of the sigmoid colon (Vercellini *et al.*, 1998), and they tend to lean on the left tube and ovary. The cysts affix to the pelvic brim via film adhesions (Vercellini *et al.*, 2000).

The peritoneal cavity is composed of one mesothelial layer containing factors such as steroids, prostaglandins, cytokines, and growth factors. Small molecules such as water and electrolytes are free to cross the membrane into the cavity

(Wang *et al.*, 1997). Elevated peritoneal fluid volume has been reported in endometriosis.

However, no single theory can explain the histogenesis and the pleomorphic manifestations of endometriosis. Retrograde menstruation has been proposed by Sampson as an aetiological factor in the causation of the disease process. Diagnostic laparoscopy has demonstrated retrograde menstruation in 90% of women (Halme *et al.*, 1983). The presence of endometrial cells in the peritoneal cavity leads to the recruitment of monocytes, which in turn elicits cytokine secretion. A local pelvic inflammatory reaction results (Cao *et al.*, 2004).

Pro-inflammatory factors such as haemoglobin and haem are released from erythrocytes. The final by-products of haemoglobin metabolism, such as haem, iron, carbon monoxide, bilirubin, and biliverdin, are biologically active compounds. Haem contains the redox generating iron molecule. Free haem has been proposed to induce lipid peroxidation, resulting in the formation of cytotoxic lipid peroxides and DNA damage (Kumar and Bandyopadhyay, 2005). The enzymes haem oxygenase-1 and haem oxygenase-2 are expressed in the peritoneal mesothelium. Together with the macrophages, these enzymes help detoxify the haem molecule (Van Langendonck *et al.*, 2002). Lipid peroxidation is one of the most destructive forms of oxidation, due to the direct effect of reactive oxygen species (ROS) on the polyunsaturated fatty acids found in the peritoneal cavity membrane (Van Langendonck *et al.*, 2002). It causes a number of detrimental effects, such as an increase in membrane permeability, loss of membrane integrity, and enzyme inactivation (Halliwell, 1994).

Endometriosis is one of the predisposing conditions associated with the development of ovarian cancer. Ness *et al.*, have proposed that both conditions are associated with underlying inflammation (Ness and Cottréau, 1999). Inflammation is linked to increased levels of oxidative stress (OS) and of pro-inflammatory factors such as cytokines and chemokines.

Oxidant/antioxidant balance

Oxidants in biological systems are, for the most part, oxygen-derived molecular species formed as intermediary products (Agarwal and Allamaneni, 2004a). Oxidants are versatile molecules well known for their ability to react with virtually all cellular components. When they are present above a critical threshold, they can induce significant structural and functional cell damage. However, concentrations of oxygen radicals below this threshold have been shown to play a physiological role in the processes of fertilization and oocyte maturation (Riley and Behrman, 1991; Agarwal and Allamaneni, 2004b).

ROS such as superoxide anion, hydrogen peroxide, and the hydroxyl radical are characterized as oxidants (Agarwal and Allamaneni, 2004a). During in-vivo studies of biological oxidation, electron leakage from the inner mitochondrial membrane has been identified as the main source of ROS (Agarwal and Allamaneni, 2004a,b). External agents such as ionizing radiation can also result in ROS generation (Agarwal and Allamaneni, 2004a,b). The unstable ROS attack most biomolecules, including lipids, proteins, and nucleic acids, resulting in various forms of cell damage.

Antioxidants represent the protective mechanisms used by cells against oxidants. ROS are neutralized by antioxidants in order to prevent OS. Stabilization of ROS occurs only after antioxidants donate electrons to the oxygen-based free radical, forcing it to change from the excited to the ground state. Enzymes such as catalase, superoxide dismutase and glutathione peroxidase/reductase are classified as antioxidants (Sikka, 2004). Non-enzymatic antioxidants include vitamin C, vitamin A, vitamin E, pyruvate, and glutathione (Sikka, 2004). Both enzymatic and non-enzymatic antioxidants are present in the female genital tract (McLaren *et al.*, 1996; Jozwik *et al.*, 1999). High concentrations of antioxidants are also found in the follicular fluid (FF) of healthy patients. In the FF, they protect oocytes from oxidation and other ROS-induced damage (Agarwal and Allamaneni, 2004a,b). In sharp contrast, patients with endometriosis have decreased concentrations of antioxidants, including enzymatic antioxidants and vitamin E (Murphy *et al.*, 1998a,b). A decrease in antioxidant components in the peritoneal cavity may be responsible for the increase in ROS seen in women with endometriosis as well in women without endometriosis (Wang *et al.*, 1997).

Oxidative stress

OS is induced when a shift in the oxidant/antioxidant balance occurs, which favours the oxidants (Agarwal and Allamaneni, 2004a). OS can have detrimental effects on female fertility by affecting ovulation, fertilization, embryo development, and implantation (Agarwal and Allamaneni, 2004b; Agarwal *et al.*, 2005). Thus, OS is considered a cause of female infertility. This is particularly notorious in cases of endometriosis (Bedaiwy and Falcone, 2003).

It is suggested that OS is caused by ROS overproduction rather than antioxidant depletion (Agarwal and Allamaneni, 2004a). Identification of the source responsible for the increase in ROS concentrations is an important factor in OS therapy (Agarwal and Allamaneni, 2004a). Once the source is identified, it should be counteracted in order to halt any further ROS production. In the case of an unidentifiable specific aetiology, antioxidant supplementation is started. Antioxidant supplementation may be beneficial to patients depending on the specific pathology of infertility (Agarwal and Allamaneni, 2004a).

ROS in peritoneal fluid

The suggested increase in peritoneal fluid ROS concentrations in patients with endometriosis has been argued in a number of studies. In 1987, Zeller *et al.* demonstrated that patients with endometriosis experienced an increase in ROS production by PF mononuclear cells. They suggested that chronic stimulation of PF macrophages induced the release of ROS (Zeller *et al.*, 1987). Since the activation of polymorphonuclear leukocytes and macrophages is known to increase ROS production, this supports their conclusion (Van Langendonck *et al.*, 2002). Bedaiwy *et al.* reported that ROS concentrations are higher in the PF of women with unexplained infertility than in fertile patients (Bedaiwy *et al.*, 2002). In a study using chemiluminescence analysis, Wang *et al.* found that women with idiopathic infertility and women with endometriosis had higher concentrations of ROS than tubal ligation control patients. This group found no correlation between the stage of

endometriosis and ROS concentrations. Overall, the increase in ROS concentrations in patients with endometriosis was not significant, suggesting that in patients with the disease, PF ROS may not directly cause infertility (Wang *et al.*, 1997). Ho *et al.* (1997) used spectrophotometric analysis to demonstrate that the total antioxidant status in patients with endometriosis is not increased.

Elevated concentrations of oxidized low-density lipoproteins are discussed in various studies, but a definitive conclusion regarding the effects of such oxidized lipoproteins on endometriosis is unattainable due to varying study outcomes. Oxidative modification has been reported in PF, endometrium, and endometriosis (Murphy *et al.*, 1998). Epitopes produced as a result of lipid peroxidation were demonstrated by immunocytochemistry in macrophage-enriched areas of both the endometrium and endometriosis implants. An increase in the oxidation of low-density lipoproteins in patients with endometriosis was demonstrated by Murphy *et al.* (1998). The oxidation process appears to be induced via lipid peroxidation and the subsequent release of aldehydes such as malondialdehyde (Van Langendonck *et al.*, 2002). An additional lipoprotein peroxidation indicator, lysophosphatidyl choline, is increased in the PF of patients with endometriosis (Murphy *et al.*, 1998). In contrast, Arumugam and Dip have found that malondialdehyde in the PF is not related to endometriosis severity (Arumugam and Dip, 1995).

Oxidative stress and endometriosis

Many studies have investigated OS markers and antioxidant status in patients with endometriosis. Inducers of OS may include erythrocytes, apoptotic endometrial cells and undigested endometrial cells in the menstrual effluent. These factors may cause activation and recruitment of mononuclear phagocytes. Activated macrophages induce OS, lipid peroxide formation, and other by-products resulting from the interaction of apolipoproteins with peroxides. OS leads to a localized pelvic inflammatory reaction resulting in increased concentrations of cytokines, growth factors, and other pro-inflammatory mediators.

Many studies have reported inconsistent results when analysing ROS concentrations in the endometrium. Therefore, it is virtually impossible to postulate a definitive conclusion regarding the role of OS in endometriosis. Ota *et al.* (1999) argue that increased OS concentrations are present due to xanthine oxidase concentrations in endometriosis patients. Xanthine oxidase is recognized as an ROS-generating enzyme. The expression of this enzyme was monitored by Ota *et al.* (1999), in the menstrual cycles of women with and without endometriosis. Women with the disease had high concentrations of ectopic and eutopic endometrial xanthine oxidase expression throughout their cycles, while women lacking endometriosis had cyclic variations of the enzyme. In previous studies, Ota *et al.* (1998) documented continuously elevated manganese and copper/zinc superoxide dismutase, as well as glutathione peroxidase, in the endometrium of women with endometriosis. Patients without the disease once again showed cyclic variations in both enzymes (Nuojuua-Huttunen *et al.*, 1999; Dokras *et al.*, 2000).

There is a cyclical expression of nitric oxide synthase (NOS) mRNA in the epithelial glands of human endometrium. Greater amounts of nitric oxide (NO) and NOS are present in the endometrium of women with endometriosis (Murphy *et al.*, 1998; Khorram and Lessey, 2002; Wu *et al.*, 2003). NOS expression in the ectopic endometrium of patients with adenomyosis is continuous throughout the menstrual cycle (Kamada *et al.*, 2000).

Omland *et al.* have also reported an increased expression of endothelial NOS in the glandular endometrium in patients with endometriosis (Omland *et al.*, 1998; Khorram and Lessey, 2002). The inducible NOS isoform was elevated in tissues of patients with endometriosis (Wu *et al.*, 2003). Endometrial development affects embryo implantation, and therefore an asynchrony between endometrial receptivity and embryo stage could impair embryo implantation and oxidative stress may result in this asynchrony. NO affects fecundity in endometriosis and adenomyosis (Nuojua-Huttunen *et al.*, 1999). Significant differences in uterine hyperperistalsis and dysperistalsis are found in patients with endometriosis compared with controls, which may be responsible for defective sperm transport and reduced fertility (Leyendecker *et al.*, 1996). The presence of lipid-protein complexes has been examined in the endometrium of patients with endometriosis. Increased lipid-protein complex modification in the endometrium has been documented by Murphy *et al.* (1998).

Free radicals and endometriosis

ROS studies

Szczepanska *et al.* (2003) reported a statistically significant decrease in peritoneal fluid superoxide dismutase and glutathione peroxidase concentrations in patients with endometriosis compared with those with idiopathic infertility and control patients. A statistically significant increase in lipid peroxide concentrations ($P < 0.039$) was found in patients with endometriosis compared with controls. Polak *et al.* (2001) reported that the total antioxidant status was significantly lower in patients with unexplained infertility (0.49 ± 0.21) than in fertile patients (0.67 ± 0.24 mmol/l, $P = 0.02$) and women with tubal infertility (0.76 ± 0.26 mmol/l, $P = 0.001$). A significant increase in PF 4-hydroxynonenal and malondialdehyde was found in patients with unexplained infertility. Plasma concentrations of lipid peroxides did not differ significantly from PF concentrations. A significant increase in lipid peroxide concentrations (3.29 ± 1.15 versus 2.34 ± 0.72 ng/ml, $P = 0.01$) in endometriosis patients was reported by Liu *et al.* (2001).

Multivariate regression analysis showed that PF tumour necrosis factor α (TNF α) could be used to differentiate patients of endometriosis from other groups of patients. ROS concentrations also reached significantly high levels in idiopathic infertility.

Increased concentrations of TBARS (thiobarbituric acid reactive substances) in serum have been associated with endometriosis, as revealed by multivariate regression analysis after adjusting for age, body mass index, smoking, and hormone use in the past 12 months. The study had an *ad hoc* power of 87% for detection of a 25% rise in TBARS with an $\alpha = 0.05$ (Jackson *et al.*, 2005).

It is significant that the important confounders were controlled for in this study.

Antioxidant enzymes are over expressed as a result of excessive free radical generation in endometriosis. Expression of the enzyme catalase in both eutopic and ectopic endometrium is increased in patients with endometriosis (Ota *et al.*, 2002). Xanthine oxidase, NOS and superoxide dismutase concentrations have also been reported to be over-expressed in endometriosis (Ota *et al.*, 1998, 1999; Polak *et al.*, 2001).

Nitric oxide studies

Although NO at physiological concentrations is beneficial as a vasodilator and paracrine factor, it can be toxic above a critical threshold and thus damage a number of biomolecules (Van Langendonck *et al.*, 2002). NO is produced by endothelial NOS located in the endometrium (Cameron and Campbell, 1998; Omland *et al.*, 1998). Cameron *et al.* demonstrated that NOS expression is increased during the menstrual cycle of women with endometriosis, suggesting that ROS may also be increased during the cycle (Cameron and Campbell, 1998). Increased generation of nitric oxide species in the PF microenvironment is associated with endometriosis, but the increasing severity of the disease may not result in a proportionate increase in the NOS radical.

NO and superoxide can react with each other to form peroxynitrite, a radical that can be considered as toxic as the hydroxyl radical (Hippeli and Elstner, 1999). Ectopic endometrial production of peroxynitrite is documented in patients with adenomyosis, a type of endometriosis found in the myometrium (Kamada *et al.*, 2000). Ho *et al.* (1997) found no evidence of increased metabolism of NO in the PF of women with or without endometriosis. The total content of nitrite and nitrate in PF has been reported to be significantly higher in patients with endometriosis (Dong *et al.*, 2001). NOS activity and the expression of inducible NOS have also been demonstrated to be higher in peritoneal macrophages from women with endometriosis. Ho *et al.* (1997) reported no significant difference in total antioxidant status and by-products of NO metabolism. In addition, women with advanced endometriosis have a significant increase in PF volume compared with controls ($P = 0.007$).

Endometriosis is found in about 20–50% of infertile women who have laparoscopy as part of their infertility workup. Increased generation of free radicals was reported to be associated with endometriosis (Bedaiwy and Falcone, 2003). There is a complex interaction of oxidative stress and cytokines, chemokines and growth factors in the peritoneal fluid environment resulting in the generation of pelvic inflammatory reaction. Ho *et al.* (1997) demonstrated that there was no significant difference in total NO production in peritoneal fluid during the early follicular phase among women with early or advanced endometriosis compared with fertile controls. The generation of cytokines secreted from endometrial cells, immune cells, or macrophages stimulates endothelial NO synthase to release NO (Omland *et al.*, 1998; Nuojua-Huttunen *et al.*, 1999; Van Langendonck *et al.*, 2002). These abnormal immune responses might eventually stimulate macrophages and/or endometrial cells to persistently produce high concentrations of NO and inhibit implantation (Kim *et al.*, 2004). NO is a free radical with deleterious

effects, and is an important bioregulator of apoptosis (Chung *et al.*, 2001). Activation of polymorphonuclear leukocytes and macrophages leads to increased production of ROS (Zeller *et al.*, 1987). An increase in number and activity of macrophages is accompanied by release of more cytokines and other immune mediators, such as NO. This was initially considered in the implication of low-grade inflammation, whereas elevated peritoneal NO concentrations are consistent with the increased number and activity of macrophages (Dong *et al.*, 2001). Elevated concentrations of NO, such as those produced by activated macrophages, can impair fertility in several ways, including changing the composition of the PF environment that hosts the process of ovulation, gamete transport, sperm oocyte interaction, fertilization, and early embryonic development (Dong *et al.*, 2001; Polak *et al.*, 2001; Szczepanska *et al.*, 2003). Studies investigating the association of NO concentrations, lipid peroxides, and ROS in PF did not find any significant difference in patients with or without endometriosis (Arumugam and Dip, 1995; Wang *et al.*, 1997). Conflicting results were obtained in studies conducted by Szczepanska *et al.* (2003). In the PF of women with endometriosis-associated infertility, the total antioxidant capacity was reduced, and the individual antioxidant enzymes such as superoxide dismutase were significantly lower. Lipid peroxide concentrations were highest amongst patients with endometriosis, suggesting a role of ROS in the development of the disease (Szczepanska *et al.*, 2003).

Apparently higher concentrations of NO were found in the ectopic endometrium compared with the eutopic endometrium in fresh endometriotic specimens, although they were not statistically significant. In addition, the expression of inducible NOS was elevated in endometrium from women with endometriosis (Wu *et al.*, 2003). Endothelial nitric oxide synthase expression was also significantly elevated in the glandular and luminal epithelium and was reported to be associated with lowered expression of $\alpha_v\beta_3$ integrin in both the epithelia (Khorram and Lessey, 2002). Volume of peritoneal fluid was reported to be significantly increased in women with advanced endometriosis (Ho *et al.*, 1997). NO concentrations in PF, peritoneal macrophage NOS activity, and peritoneal macrophage NOS-2 protein expression were studied in women with endometriosis-associated infertility. Peritoneal macrophages from women with endometriosis-associated infertility expressed higher concentrations of NOS-2, had higher NOS enzyme activity, and produced more NO in response to immune stimulation *in vitro* (Osborn *et al.*, 2002). The high concentrations of NO adversely affected spermatozoa, embryos, implantation and oviductal function. This suggests that reducing NO production in PF or blocking NO effects may improve fertility in women with endometriosis (Osborn *et al.*, 2002). Generation of peroxynitrite by ectopic endometrium was demonstrated in patients with adenomyosis. Expression of endothelial and inducible NOS and peroxynitrite generation were markedly reduced after gonadotrophin-releasing hormone (GnRH) agonist therapy, supporting their potential role in the pathophysiology of adenomyosis (Kamada *et al.*, 2000). Therapeutic modalities such as GnRH agonists modulate OS concentrations, as serum NO concentrations are suppressed by GnRH agonists and up-regulated by gonadotrophin stimulation during controlled ovarian stimulation in female partners from couples with male factor infertility (Ekerhovd *et al.*, 2001). Maximal concentrations were measured at the time of ovulation in the same study. Elevated NO production was not demonstrated in patients with ovarian hyperstimulation.

Two studies reported that PF from patients with endometriosis contains increased concentrations of NO (Dong *et al.*, 2001; Osborn *et al.*, 2002). It has also been postulated that ROS may have a role in the formation of adhesions associated with endometriosis (Portz *et al.*, 1991). Altered folliculogenesis resulting in impaired oocyte quality has been proposed to be a cause of subfertility associated with endometriosis. OS may impair oocyte quality. Concentrations of an OS marker, 8-hydroxy-1-deoxyguanosine index, were higher in patients with endometriosis than in patients with other causes of infertility, such as tubal, male factor or idiopathic causes (Seino *et al.*, 2002). A 6-fold increase in the concentrations of 8-hydroxy-1-deoxyguanosine and lipid peroxides was demonstrated in ovarian endometriomas compared with normal endometrial tissue (Kao *et al.*, 2005).

Earlier studies found that the expression of NOS is elevated in patients with endometriosis. Now strong evidence has been found that a common polymorphism of exon 7 at nucleotide 894 in the endothelial NOS (*eNOS*) gene was associated with endometriosis (Zervou *et al.*, 2003). Hence, variations in the expression of the *eNOS* gene may be involved in endometrial angiogenesis and thus modulate the process of endometriosis.

Endometriosis has been proposed to be related to a localized pelvic inflammatory reaction. There is a complex interplay of OS, cytokines, and angiogenesis in the pathophysiology of endometriosis. Increased expression of endothelial NOS has been reported throughout the menstrual cycle in the endometrium of women with endometriosis (Cao *et al.*, 2004).

Molecular genetic pathways and oxidative stress in endometriosis

Gene deletion of mitochondrial DNA resulting in rearrangement of mitochondrial DNA was reported in endometriotic tissue (Kao *et al.*, 2005). Differences in gene expression levels between ectopic and eutopic endometria were determined comprehensively, and Wu *et al.* (2006) reported that 904 genes were differentially expressed. Wu *et al.* reported the differential expression of glutathione-S-transferase gene family, which are involved in the metabolism of the potent antioxidant glutathione. The cellular response, which includes proliferation and angiogenesis, to oxidative stress may also be determined by differential gene expression. Newer therapeutic approaches may be designed in future based on the gene profiling characteristics.

Studies of endometriosis in animal models

The pathogenesis of endometriosis has been studied in a rat model. Rats were utilized for induction of endometriosis using a surgical auto-transplantation technique (Uchiide *et al.*, 2002). Induction of endometriosis has been studied both in primate and non-primate animal models. Primates can develop the disease spontaneously, and in non-primates, endometriosis can be induced by autologous transplantation of endometrium or human endometrium. On microscopic examination, stromal tissues of uterus attached to the peritoneum show proliferation

and infiltration of mast cells, eosinophils, plasma cells, lymphocytes, and macrophages. The rat lesions induced were similar to those in endometriosis patients with a hypersensitivity reaction induced in the peritoneal stroma attached endometrial epithelium. This supports the hypothesis that endometriosis is a local pelvic inflammatory disorder.

Antioxidants inhibit the induction of endometriosis in the rabbit (Portz *et al.*, 1991). Immunomodulators such as loxoribine and pentoxifylline have beneficial effects in animal endometriosis models. Hyperactivation of macrophages induces subfertility associated with endometriosis. The action of macrophages was shown to be reversed with the immunomodulator pentoxifylline (Steinleitner *et al.*, 1991a,b). Epithelial and stromal components of endometriosis were both shown to regress with the drug loxoribine in a rat model (Keenan *et al.*, 1999). Stockemann *et al.* demonstrated the anti-proliferative and antioxidant effects of antiprogesterins in a rat model of surgically induced endometriosis (Stoekemann *et al.*, 1995). Verapamil, a calcium channel blocker, is known to reverse macrophage activation. In an in-vitro study, PF from patients with endometriosis was incubated with the immunomodulators verapamil and pentoxifylline. There was a significant increase in the phagocytic activity of macrophages. Periovarian treatment of hamsters with the immunomodulator verapamil was demonstrated to be better than conventional treatment for endometriosis-associated subfertility (Steinleitner *et al.*, 1991). Thus, research on endometriosis and OS can benefit from both human and animal studies.

Antioxidant systems in endometriosis

Antioxidants and OS can modulate the growth of endometrial stromal cells, as shown in an in-vitro study (Foyouzi *et al.*, 2004). Foyouzi *et al.* did not find any differences in the response of endometrial cells from the endometriosis and the non-endometriosis group of patients. An animal study conducted by Portz *et al.*, demonstrated that catalase and superoxide dismutase directly injected into the rabbit peritoneal cavity prevented the formation of intraperitoneal adhesions at foci of endometriosis (Portz *et al.*, 1991).

The role of antioxidants in endometriosis is controversial, mainly because of contradictory views expressed by various authors. Antioxidant systems are known to protect tissues from the adverse effects of free radicals. A lower total antioxidant potential has been found in the PF of infertile women with endometriosis compared with women with idiopathic infertility (Szczepanska *et al.*, 2003). Portz *et al.* suggested that antioxidants protect against endometriosis. They showed that a combination of enzymes that scavenge ROS reduced endometrioma adhesions (Portz *et al.*, 1991). In contrast, Arumugam and Yip were unable to identify a causal relationship between antioxidants and endometriosis (Arumugam and Dip, 1995). Polak *et al.* has also reported a lower total antioxidant potential in the PF of patients with idiopathic infertility (Polak *et al.*, 2001). Therefore, the ever-present question of whether OS is involved in the pathogenesis of infertility in endometriosis patients has yet to be resolved.

Glutathione peroxidase and superoxide dismutase (SOD) are

antioxidant enzymes that prevent the detrimental biological effects of OS. Both enzymes scavenge ROS and inhibit the production of the hydroxyl radical. Three types of SOD have been identified: SOD-1, SOD-2, and extracellular SOD. SOD-1 is located in the cytoplasm and contains both copper and zinc. SOD-2 is found in the mitochondrial matrix and contains manganese. Extracellular SOD is much like SOD-1 in that it contains copper and zinc, but it is found on the cell surface. A study conducted by Szczepanska *et al.* documented a statistically significant lower concentration of SOD in infertile women with endometriosis (Szczepanska *et al.*, 2003). The physiological role of SOD is to scavenge superoxide anions in order to protect cells from this radical. A mouse embryo study conducted by Noda *et al.* demonstrated that the proliferation arrest of fertilized oocytes might be reversed *in vitro* by the addition of SOD to the culture medium, suggesting that oxygen radicals might be involved in in-vivo embryo development (Shiotani *et al.*, 1991). An additional animal study conducted by Portz *et al.* demonstrated that catalase and SOD, when directly injected into the rabbit peritoneal cavity, prevented the formation of intraperitoneal adhesions at endometrial foci (Portz *et al.*, 1991). Liu *et al.* (2001) found that SOD concentrations in women with endometriosis-associated infertility were statistically significantly lower than concentrations in fertile controls. Such results suggest that ROS are involved in the pathogenesis of infertility documented in endometriosis patients. On the other hand, Polak *et al.* (2000) found no difference in PF SOD activity when studying women with endometriosis-associated infertility and other infertility causes.

Antioxidant therapy in endometriosis

Antioxidant therapy with 1200 IU of vitamin E and 1 g of vitamin C for a period of 2 months resulted in a decrease in the inflammatory markers monocyte chemoattractant protein-1, RANTES (regulated on activation and normally T cell expressed and presumably secreted), and interleukin-6 in PF (Santanam *et al.*, 2003). The serum concentrations of these markers did not show any changes, suggesting that endometriosis is a disease associated with localized pelvic inflammation. Vitamin C and E therapy also reduced pelvic pain in women with endometriosis (Kavtaradze, 2003). The results of these studies lack power due to the small number of patients enrolled in them.

Activation of macrophages can be assessed by measuring transferrin receptor expression (CD71). In two groups of patients with endometriosis (one group received GnRH analogue and the other served as a control), Matsushima *et al.* reported that CD71 expression was only detected in peritoneal and tubal macrophages from the control patients (Matsushima *et al.*, 2002). According to one hypothesis, reducing the activation of macrophages will lead to a decrease in ROS production. GnRH analogues may thus indirectly reduce OS.

Pentoxifylline, a phosphodiesterase inhibitor, has been investigated in the treatment of endometriosis. A small, randomized pilot study reported a 31% pregnancy rate in the group given pentoxifylline orally, compared with 18.5% in the placebo group. This study indicated that there was a trend towards higher pregnancy rates in the treated group, although the results did not reach statistical significance.

In an in-vitro study, RU 486 was found to exert an inhibitory effect on endometrial cell growth and that this effect was mediated, at least in part, through its antioxidant properties. Another anti-proliferative agent, Lazaroid U74, 500A was found to have a direct inhibitory effect on endometrial cell growth. Many progesterone antagonists and progesterone receptor modulator drugs have been reported to have anti-proliferative activities, which may be beneficial to patients with endometriosis (Spitz, 2003).

Although the antioxidants vitamins C and E and drugs with antioxidant effects such as RU 486 have been shown to have some beneficial effects in patients with endometriosis, a limited number of trials have been conducted, and therefore these results need to be confirmed in larger randomized clinical trials.

Endometriosis and fertilization

As indicated previously, the presence of increased concentrations of ROS in the oviductal fluid could have detrimental effects on oocyte and spermatozoa viability and the process of fertilization and embryo transport in the oviduct. In addition, the concomitant presence of activated neutrophils and macrophages and pro-inflammatory factors in the oviductal fluid could significantly amplify ROS production by foci of endometriosis. Previous studies found that exposure of ROS-producing immature spermatozoa to activated leukocytes resulted in a significant increase in ROS production by these spermatozoa to concentrations that were comparable with those produced by activated leukocytes (Saleh and Agarwal, 2002). This increase in ROS production could result in oxidative damage to the sperm plasma and acrosomal membranes, leading to loss of motility and the ability of spermatozoa to bind and penetrate the oocyte respectively, and DNA damage leading to failed fertilization, reduced embryo quality, pregnancy failure, and spontaneous abortion. In a recent study conducted on patients undergoing IVF, it has been shown that exposure of spermatozoa to sera from non-pregnant women resulted in a significant increase in sperm DNA fragmentation compared with exposure to sera from pregnant women (Bouma *et al.*, 2004). This suggests that toxic factors present in the sera from non-pregnant women similar to those found in the oviductal fluid of patients with endometriosis could damage spermatozoa.

It has been recently reported that the anti-inflammatory drug infliximab, a monoclonal antibody that blocks TNF α , had a protective effect on TNF α -induced sperm damage *in vitro*. This included loss of motility, membrane damage and DNA fragmentation (Said *et al.*, 2005). Therefore, the use of anti-inflammatory drugs may protect spermatozoa from pro-inflammatory factors and pro-inflammatory-induced OS in patients with endometriosis.

Another piece of evidence favouring the hypothesis that increased concentrations of ROS and pro-inflammatory factors in the oviductal fluid may have a deleterious effect on the process of fertilization is related to the reported beneficial effects of hysterosalpingography (HSG) on pregnancy rates in women with endometriosis (Johnson *et al.*, 2004). The injections of radiological contrast through the oviduct during the HSG procedure may help 'flush out' these toxic factors as well as permeabilize any obstructions. This could have

beneficial effects on gamete viability and gamete and embryo transport. Many of the therapeutic interventional studies have also provided evidence of the role of OS in endometriosis.

Conclusions

Endometriosis is a wide-spectrum disease that includes a number of pathophysiological processes. Although it is a relatively benign disease, it has invasive capabilities. Endometriosis is a dynamic disease that can be progressive. Recurrence of the disease is known to occur. Of the several hypotheses that have been proposed to explain the pathophysiology of endometriosis, OS is one of the leading theories. There is cumulative evidence in animal and human studies that show OS is one of the main processes in the pathogenesis of endometriosis. However, there is a lack of uniformity among the different studies in terms of OS markers, types of tissues and fluids studied, and the severity of the disease. The balance between menstrual effluent, OS, and antioxidants may determine whether the disease is static or progressive or whether it recurs after suppressive therapy. Few studies have investigated multiple markers of OS and antioxidant concentrations. The studies reviewed were predominantly case-control and observational studies. Many of these studies lack statistical power because of small patient numbers. OS and inflammation are closely linked and have been proposed to play a role in the aetiology of both ovarian cancer and endometriosis. The beneficial effects of antioxidant therapy and immunomodulators need to be investigated in larger studies with adequate statistical power.

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