Oxidative Stress in Unexplained Female Infertility

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Introduction

Unexplained infertility is one of the controversial subjects in infertility on which agreement is rarely found among practitioners. It is a term used to define 30–40 % of couples in whom standard investigations including semen analysis, tests of ovulation and tubal patency have failed to detect any gross abnormality [1]. Couples with unexplained infertility suffer from both diminished and delayed fecundity. The possible underlying etiologies are defective endometrial receptivity, impaired oocyte quality, premature ovarian failure, minimal and mild endometriosis, tubal disease, pelvic adhesions, immunological and endocrinological abnormalities, and oxidative stress [2].

A couple is referred for infertility investigation if they are not able to conceive within a year. The diagnosis of unexplained infertility may be frustrating because if there is no explanation for infertility, there is no effective treatment. The prognosis is worse if the duration of infertility exceeds 3 years and female partner is >35 years of age [3]. Treatment has been indicated if the duration is more than 2 years or the female partner is >35 years [3, 4] of age.

Oxidative stress has been known to play a key role in the pathogenesis of subfertility in both males and females [5]. The adverse effects of oxidative stress on sperm quality and functions have been studied in detail [6]. Although it has been associated with female reproductive disorders such as endometriosis and polycystic ovarian syndrome (PCOS), the impact of oxidative stress on unexplained female infertility has not been adequately studied. The aim of the review is to investigate the possible relationship between underlying mechanisms that may be associated with unexplained infertility and oxidative stress by using the currently available literature.

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What Is Oxidative Stress?

Biological systems contain an abundant amount of O_2. Free radicals are often generated from O_2 and partially from normal metabolic processes in the body. They are unstable and highly reactive due to unpaired electrons that are capable of initiating an uncontrolled cascade of chain reactions, resulting in cellular damage and disease [7]. There are two major types of free radical species: reactive oxygen species (ROS) and reactive nitrogen species (RNS).The three major types of ROS are superoxide (O_2^-), which are formed when electrons leak from the electron transport chain; hydrogen peroxide (H_2O_2), resulting from the dismutation of superoxide or directly from the action of oxidase enzymes, and hydroxyl ($HO^\bullet$), a highly reactive species that can modify purines and pyrimidines and cause strand breaks that result in DNA damage.

The controlled production of ROS plays a role in the production of physiological reproductive processes such as hormone signaling, oocyte maturation, folliculogenesis, tubal function, ovarian steroidogenesis, cyclical endometrial changes, and germ cell function. Oxidative stress occurs when ROS overwhelm antioxidant capacity. ROS extensively damage cellular organelles, including mitochondria, nuclear and mitochondrial DNA, and cell membrane, leading to cellular demise [8, 9]. ROS may also impact signaling pathways, transcription factors, and epigenetic mechanisms and cause a reduction in oocyte, embryo quality and implantation [6, 10].

Energy from adenine triphosphate (ATP) is essential for gamete functions and any disturbance in mitochondrial functions can lead to altered generation of ATP. Mitochondria are major sites of ROS production and increased generation of ROS can affect functions of the mitochondria in oocytes and embryos.

RNS include nitric oxide (NO) and nitrogen dioxide as well as nonreactive species such as peroxynitrite, nitrosamines, and others. NO, an important RNS that is present in the body, is specifically synthesized by nitric oxide synthase

G. L. Schattman et al. (eds.), *Unexplained Infertility,* DOI 10.1007/978-1-4939-2140-9_14,

(NOS) during the conversion of L-arginine to L-citrulline. Excess NO is toxic and has an unpaired electron, making it a highly reactive free radical that can damage proteins, carbohydrates, nucleotides, and lipids. It contributes to cell and tissue damage, low grade, sterile inflammation, and adhesions with other inflammatory mediators [11]. NOS have been known to generate H_2O_2, superoxide, and NO. Superoxide reacts with NO, resulting in increased generation of peroxynitrite and cell toxicity. The effects of NO are proposed to be mediated through cyclic guanosine monophosphate (cGMP) as a second messenger or by generation of ROS resulting from interaction of NO with superoxide radicals [12].

Intracellular hemostasis is maintained by a balance between pro-oxidant compounds and antioxidants. Antioxidants have the ability to oppose the effects of pro-oxidants by hindering ROS production, scavenging ROS, and repairing cell damage caused by ROS. Enzymatic antioxidants include superoxide dismutase, catalase, and glutathione peroxidase. Nonenzymatic antioxidants are vitamins C and E, taurine, hypotaurine, cysteamine, and glutathione. Some agents such as lycopene, metallothionein, and bilirubin also have antioxidant properties.

Antioxidants can be found in various cell compartments. For example, Mn–SOD is localized in the mitochondria, whereas Cu–Zn–SOD is mainly localized in the cytoplasm. Enzymatic antioxidant defenses have been found in mammalian embryos and oocytes [13, 14], and nonenzymatic defenses in tubal [14] and follicular fluids [15]. Increased levels of antioxidants have been shown in normal pregnancy [16, 17], whereas loss of antioxidant defenses have been observed in patients with recurrent abortion as a result of their increased consumption [18, 19]. The lower antioxidant levels could aggravate pro-oxidant injury in endothelial cells, altering prostacyclin–thromboxane balance and may contribute to preeclampsia or abortion [20]. Glutathione peroxidase expression was increased and selenium levels were reduced in patients with diabetes and spontaneous abortion [16].

Redox Cell Signaling

Redox reactions can be defined as oxidation or reduction of oocyte and embryo metabolism by electron transfer mechanisms [6]. Oocytes are protected from oxidative damage by antioxidants such as catalase, SOD, glutathione transferase, paraoxanase, and heat shock proteins [21].

The deleterious effects of ROS are summarized as follows:

1. Opening of ion channels: Elevated levels of ROS release Ca^{+2} from endoplasmic reticulum resulting in mitochondrial permeability, so the mitochondrial membrane potential becomes unstable and ATP production ceases.
2. Lipid peroxidation: It occurs in polyunsaturated fatty acid side chains. These chains react with O_2 creating the peroxyl radical, which can obtain H^+ from another fatty acid, creating a continuous reaction. Vitamin E can break this chain reaction due to its lipid solubility and hydrophobic tail.
3. Protein modifications: Amino acids are targets for oxidative damage. Direct oxidation of side chains can lead to the formation of carbonyl groups.
4. DNA oxidation: Mitochondrial DNA is susceptible to ROS due to the presence of O_2^- in the electron transport chain, lack of histone protection, and absence of repair mechanisms.

ROS may react with other molecules to disrupt many cellular components and processes. ROS target the pathway and also act as second messengers in some reactions [22]. ROS are known to effect ovulation and implantation [23].

Nuclear factor-kappa B (NF-KB) is a transcription factor that plays a crucial role in inflammation, immunity, cell adhesion, invasion, cellular proliferation, apoptosis and angiogenesis [24]. Oxidative stress is known to be a potent activator of NF-KB [24]. Induced NF-KB activation then leads to expression of numerous proinflammatory genes such as cytokines, which may provide positive feedback to the pathway [25].

One of the most signaling pathways in the body is mitogen-activated protein kinases (MAPK). MAPK pathways are important in gene transcription mechanisms in response to oxidative stress. Their signaling cascades are controlled by phosphorylation and dephosphorylation of serine and/or threonine residues. It contributes to increased actions of receptors of tyrosine kinases, protein tyrosine kinases, cytokines, and growth factors [26, 27]. c-jun N-terminal kinases (JNK) and p38 pathways can also be activated by ROS. The addition of H_2O_2 to this cascade can disrupt the complex and promote phosphorylation [28, 29].

Oxidative Stress and the Ovary

ROS are a double-edged sword; they not only serve as key signal molecules in physiological processes but also have a role in pathological situations involving female reproductive tract. Oxidative stress is important in ovarian germ cell and stromal cell physiology. A cohort of oocytes begins to grow and develop in the ovary each month. Hypoxia of the granulosa cells is a normal event during the growth of ovarian follicles [30]. Hypoxic or anoxic conditions reduce ROS and increase antioxidant activity [31]. Oxygen deprivation stimulates follicular angiogenesis, which is important for growth and development of the ovarian follicle. Impairment of angiogenesis within ovarian follicles contributes to follicular atresia. Meiosis I resumes in the dominant follicle that

is regulated by ROS. Antioxidants positively affect dominant follicle selection [32].

It has been found that cyclical ROS production and diminished antioxidant activity may contribute to oophoritis associated with autoimmune premature ovarian failure [33].

Meiosis II is promoted by antioxidants [33]. Granulosa and luteal cells respond negatively to ROS and affect MII progression, leading to diminished gonadotropin, and antisteriodogenic actions, DNA damage, and inhibited ATP progression in both humans and rats [33]. Glutathione, an antioxidant has been identified as critical for oocyte maturation, particularly in cytoplasmic maturation required for preimplantation development and formation of male sperm pronucleus [34, 35]. Beta carotene, another antioxidant, has been also recognized to enhance cytoplasmic maturation [36].

The steroid production in the leading follicle causes an increase in P450. Overexposure of the ovary to H_2O_2 causes the LH receptor to uncouple from adenylate cyclase, impairing protein synthesis and cholesterol utilization by mitochondrial P450 side chain cleavage, most likely because of impaired production of steroidogenic acute regulatory protein (StAR) [33]. StAR is responsible for moving cholesterol to the inner mitochondrial membrane where $P450_{scc}$ converts cholesterol to pregnenolone [37]. Lecithin-cholesterol acyltransferase (LCAT) plays an important role in cholesterol transport and follicular synthesis to estrogen. High follicular fluid LCAT is positively associated with ascorbate and alpha tocopherol accumulation and the presence of antioxidants in follicular fluid protect LCAT from oxidative damage and steroidogenesis [38].

Oxidative stress is important in ovulation [39]. LH surge is essential for ovulation that is triggered by oxidative stress [40]. Follicular ROS promote apoptosis and glutathione and FSH counterbalance its action. Estrogen increases in response to FSH, triggering the generation of catalase in the dominant follicle, thus avoiding apoptosis [33].

Corpus luteum is produced after ovulation and ROS are also produced in the corpus luteum. Cu, Zn-SOD decreases and ROS increases during the regression of corpus luteum. This activity parallels the change in progesterone levels. Complete disruption of the corpus luteum causes a substantial decrease of Mn-SOD in the regressed cell and cell death is imminent [40]. Cu, Zn-SOD is related to progesterone production and Mn-SOD protects luteal cells from oxidative stress [33].There are also some other oxidative stress markers such as superoxide dismutase, Cu–Zn superoxide dismutase, Mn superoxide dismutase, glutathione peroxidase, γ glutamyl synthetase, and lipid peroxides ovarian physiology [41–43].

Glutathione concentrations are higher in mature, metaphase hamster and mouse oocytes [44]. Antioxidants aid in meiotic spindle formation in mature MII oocytes [44]. Exposure to oxidative stress before fertilization disrupts the meiotic spindle and increases the risk of abnormal zygote formation. If there is an adequate antioxidant defense, O_2^- does not affect gamete fusion [45].

Oxidative Stress and Oocyte and Embryo Quality

Oxidative stress was increased in repeated ovarian stimulation that leads to mitochondrial DNA mutation and decreased oocyte quality [46]. Lipid peroxides and 8-hydroxydeoxyguanine, a marker of DNA damage, were increased between the first and sixth cycles [46]. It has been suggested that timing antioxidant administration may have an effect on the number and quality of ovulated oocytes as assessed by morphological appearance and chromosomal distribution in female mice [47, 48]. Animals receiving antioxidant supplements showed an increased number of normal MII oocytes compared with the control group and decreased percentage of apoptotic oocytes [48]. Low intrafollicular oxygenation has been associated with decreased oocyte developmental potential as shown by increasing frequency of oocyte cytoplasmic defects, alterations in spindle morphology, impaired cleavage, and abnormal chromosomal segregation in oocytes [49]. It has been reported that high ROS follicular fluid concentrations were correlated with poor oocyte quality and diminished embryo quality [50]. High concentrations of follicular ROS are associated with compromised IVF outcomes [51, 52]. Moreover, selenium-dependent glutathione peroxidase activity and total antioxidant capacity (TAC) in follicular fluid were positively associated with fertilization rates [53, 54].

Oxidative stress is also associated with decreased embryo quality, increased embryo fragmentation, resulting from increasing apoptosis [55]. Melatonin, another antioxidant, has positive effects on oocyte quality and embryo development [56]. High levels of ROS may impair intracellular milieu and lead to disturbed metabolism [15, 57]. Oxidative stress-mediated damage of macromolecules plays a role in fetal embryopathies. Folate deficiency may lead to elevated homocysteine levels. Homocysteine was negatively associated with embryo quality on culture day 3 [58] and homocysteine-induced oxidative stress may cause some fetal abnormalities such as neural tube defects and cleft palate [59]. Higher day 1 ROS levels in culture media were associated with delayed embryonic development, high fragmentation, and development of morphologically abnormal blastocysts after prolonged culture. A significant correlation was reported between elevated ROS levels in day 1 culture media and lower fertilization rates in patients undergoing intracytoplasmic sperm injection (ICSI) [60]. Lower ROS levels were associated with higher fertilization rates, indicating the physiological relevance of low levels of ROS. Clinical pregnancy rates were also higher when antioxidant supplements were added to culture media [61].

Oxidative Stress and Aging Oocyte

ROS increase with age that may contribute to follicular atresia and decline in the number and quality of oocytes [62, 63]. It has been reported that prolonged exposure of aged oocytes to ROS negatively affects calcium hemostasis and impairs Ca^{+2} oscillation-dependent signaling and causes decline in oocyte developmental ability [64]. Oxidative stress damages telomeres and accelerates telomere shortening [65, 66]. Telomere shortening and dysfunction may lead to defects in meiosis, fertilization, and embryo development [67]. In another study, it has been found that follicular fluid catalase and glutathione transferase were lower in older women compared with young ones [68]. Studies have demonstrated that N-acetyl-L-cysteine and vitamin E protect the ovary from aging [69, 70].

Oxidative Stress and Endometrium

ROS are also found in endometrium. They are produced in stromal cells as byproducts of normal metabolism [71]. Intracellular sources of ROS are mitochondrial electron transport system, endoplasmic reticulum, nuclear membrane electron transport systems, and plasma membrane [71]. SOD is highly expressed in glandular epithelial cells and stromal cells in endometrium that plays an important role in regulation of endometrial function [72].

There is a close relationship between SOD, ROS, and PGF2α in the regulation of menstruation. In human endometrium, SOD activities decrease and ROS increase in late secretory phase, just before menstruation [72]. ROS trigger the release of PGF2α production and COX-2 mRNA expression and Cu, Zn-SOD activities decline by withdrawal of ovarian steroids in human endometrial cells [73]. The increases of PGF2α production and COX-2 mRNA expression were completely suppressed by N-acetyl-L-cysteine [74].

Withdrawal of ovarian steroids activates NF-KB via ROS which stimulate the COX-2 and PGF2α in human endometrial stromal cells [74]. NF-KB signaling pathway is present in human endometrium [74]. The gene promoter human COX-2 has a binding site for NF-KB and it has been reported that COX-2 expression is regulated by NF-KB [75, 76].

When implantation is successful and progesterone levels and Cu, Zn-SOD activities are high, ROS generation and PGF2α production is suppressed. In early pregnancy, ROS are low and Cu, Zn-SOD activities are high in the decidua [72]. On the other hand when pregnancy does not occur, the decline of ovarian steroid levels induces the decrease in Cu, Zn-SOD expression in endometrial stromal cells which stimulate PGF2α production by ROS. PGF2α causes endometrial shedding via vasoconstriction.

Decidualization of stromal cell is necessary for successful implantation. Cu, Zn-SOD, and Mn-SOD were found in decidualized stromal cells and in the endometrium of the patient [72]. Decidualization promotes production of many bioactive substances such as growth factors, cytokines, and adhesion molecules. This increase in metabolism stimulates generation of superoxide radicals in the mitochondria. Antioxidants are crucial in eliminating superoxide radicals. Blockage of Mn-SOD induction causes oxidative stress-induced cell death in human endometrial stromal cells [77]. PGF2α were lower and Cu, Zn-SOD activities were higher in the decidua of normal pregnancies compared with failed pregnancies that are accompanied with uterine bleeding and contractions [78]. Cu, Zn-SOD may contribute to uterine quiescence by preventing the accumulation of ROS leading to PGF2α synthesis and uterine contraction.

Oxidative Stress and Tubal Disease

The diagnostic accuracy of hysterosalpingography (HSG) in defining normal tubal physiology and anatomy has been questioned [79]. Consensus stated that HSG is less accurate in detecting and evaluating tubal disease than laparoscopy [80, 81] and is especially poorly suited to assess distal tubal disease and peritubal disease [82, 83]. Routine HSG misses at least one anatomical or physiological tubal abnormality in 84 % of the cases [84]. Thus, undiagnosed tubal disease may be associated with unexplained infertility.

The oviduct is the first site contact with the early embryo and has the potential to contribute important factors that affect fertility. Oviduct provides an optimal environment for gamete maturation, fertilization and early embryonic development. It is an active organ that maintains and modulates the fluidic milieu for sperm capacitation, fertilization, and early embryonic development [85–87]. The environmental and metabolic stimuli from the oviduct may have a significant effect on embryonic development. Some growth factors such as insulin-like growth factor, vascular endothelial growth factor, and nitric oxide synthase have been identified as important regulatory factors of oviductal motility and embryo transport [88–91]. NO plays a role as a mediator of PGF2a-induced contractility and is important for secretory functions in the oviduct [92]. It has been reported that *Chlamydia trachomatis* induces an inflammatory response and leads to tubal epithelial destruction and functional impairment caused by high NO output mediated by inducible NOS (iNOS) [93]. Oxidative stress causes infection-induced immune reaction and inflammation-induced tissue lesions [94, 95]. Heat shock proteins (HSPs) are stress proteins that are closely associated with oxidative stress and inflammation [96]. It has been suggested that HSP60 may be involved in

the pathogenesis of tubal factor infertility following *C. trachomatis* infection [97]. HSP 60 and 70 contribute to inflammation via anti-inflammatory cytokines such as IL-10 and IL-12 in the fallopian tube resulting in chronic salpingitis with tubal occlusion [98, 99].

Oxidative Stress and Pelvic Adhesions

Adhesions may be one of the etiologic factors in unexplained infertility. Pelvic pathology was found during laparoscopy in 83.4% patients with unexplained infertility in a study [100]. Adhesions were found in 48.4% of them [100]. Moreover, the sensitivity of HSG in detecting peritubal adhesions has been reported to be 34–75% [101]. Twenty-one percent of adnexal adhesions and pelvic endometriosis were identified during surgery in spite of normal HSG [102]. The prevalence of peritubal adhesions was suggested to range from 8.8 to 29% [103, 104].

Adhesiogenesis is a complex interaction of cellular components involved in inflammation and wound repair. It has been reported that ROS are associated with adhesion formation [105]. Acute oxidative stress in the peritoneum subsequently induces mesothelial cell loss or dysfunction, peritoneal fibrosis, and intra-abdominal adhesion formation [106]. Accumulation of free radicals may result in more collagen synthesis through fibrogenic processes such as transforming growth factor beta (TGF-β) activation and lipid peroxidation [107]. A positive correlation between oxidative stress and the severity of peritoneal adhesions has been demonstrated [108]. It has been also known that endometriosis, another risk factor for pelvic adhesion, is closely associated with endometriosis [6].

It has been reported that antioxidants such as methylene blue, melatonin, vitamin E, and alpha lipoic acid were able to decrease peritoneal adhesions [108, 109]. Peritoneal TAC scavenges free radicals and protects peritoneal tissue from oxidative damage. Furthermore, antioxidants have been shown to increase tissue plasminogen activator gene expression in endothelial cell cultures and to increase plasma fibrinolytic activity in humans [110–112].

Oxidative Stress and Endometriosis

Oxidative stress has been implicated in pathophysiology and progression of endometriosis [113–115]. There are several hypotheses that may explain the relationship between oxidative stress and endometriosis. Erythrocytes yield proinflammatory factors hemoglobin and heme, containing the redox generating iron molecule [116]. Oxidative stress precipitates endometriosis and tissue growth may result from iron, macrophages, and environmental contaminants such as polychlorinated biphenyls [117]. The peritoneal fluid of the patients have been found to contain high concentrations of malondialdehyde (MDA), proinflammatory cytokines (IL-6, TNF-alpha, and IL-beta), angiogenic factors (IL-8, VEGF), monocyte chemoattractant protein-1 [118] and oxidized LDL (ox-LDL) [119] and reduced levels of antioxidants such as total antioxidant capacity (TAC) and SOD [114, 120]. Proinflammatory and chemotactic cytokines play an important role in the recruitment and activation of phagocytic cells, which produce ROS and RNS [118].

Lipid peroxidation and oxidative stress have been demonstrated by increased levels of 8-iso-prostaglandin F2-alpha (8-iso-PGF2-alpha) [121, 122]. It has been reported that 8-iso-PGF2-alpha in both urine and peritoneal fluid of patients was significantly elevated [123].

Circulating levels of oxidative stress from other sources such as endometrium and ectopic endometrial implants may also contribute to the pathogenesis of endometriosis. Increased lipid–protein modification that contributes to high lipid peroxide concentrations has been shown in the endometrium of the patients with endometriosis [114, 120]. Lipid peroxidation was present in macrophage-enriched areas of both the endometrium and endometriosis implants [124]. High levels of antioxidants inhibit the proliferation of endometrial stromal cells and moderate levels of oxidative stress promote endometrial stromal cell proliferation [125].

NO was also increased in peritoneal fluid and the endometrium of the women with endometriosis [12]. Elevated levels of NO, as generated by activated macrophages, can disrupt fertility in variable ways, including altering the composition of the peritoneal fluid environment that affects ovulation, gamete transport, sperm oocyte interaction, fertilization, and early embryonic development [12]. Increased NO and NOS expression may lead to endometrial receptivity defects and hinder embryo implantation. Elevated levels of oxidative stress in oviductal fluid might have adverse effects, impairing oocyte and spermatozoa viability, fertilization, and embryo transport in women with endometriosis [105]. Oxidative stress may lead to damage to sperm plasma and acrosomal membranes, impairs motility, and hinders the ability of spermatozoa to bind to and penetrate the oocyte. DNA damage as the result of oxidative stress may contribute to failed fertilization, reduced embryo quality, failure of pregnancy and spontaneous abortion.

Immunological Infertility and Oxidative Stress

Many types of antibodies have been implicated in the pathophysiology of unexplained infertility. Antiovarian, antispermatozoal, and anticardiolipin antibodies were demonstrated

in women with unexplained infertility [126, 127]. It has been reported that inadequate maternal immunosuppression might cause embryo rejection in that group [128]. The prevalence of celiac disease is also higher in these women compared with fertile women [129]. Elevated anti-*C. trachomatis* antibodies can be detected in more than 70% of women with tubal occlusion [130]. *C. trachomatis* has a direct cytotoxic effect on the mucosa of the fallopian tube, resulting in loss of microvilli [131]. Permanent tubal damage is predominantly a consequence of a host immune response to persistent or repeated infection. Antiphospholipid antibodies are associated with thrombosis and infarction in the placenta. They inhibit the release of hCG from human placental explants, block in vitro trophoblast migration, invasion, and multinucleated cell formation, inhibit trophoblast cell adhesion molecules, and activate the complement on the trophoblast surface inducing an inflammatory response [132]. Antisperm and antiovarian antibodies may have an adverse effect on fertilization, early embryonic development, and implantation [132]. Between 10 and 30% of women with premature ovarian failure have a concurrent autoimmune disease; the most commonly reported one is hypothyroidism. It also has a close relationship between myasthenia gravis, systemic lupus erythematosus, rheumatoid arthritis, and Crohn's disease.

It has been shown that anticardiolipin antibodies were positively correlated to plasma levels of F2-isoprostanes, sensitive markers of lipid peroxidation [133]. Paraoxonase-1 (PON-1), an antioxidant, has been found to be reduced and inversely correlated with anticardiolipin antibodies and directly with total antioxidant status [134]. Increased oxidative stress may be involved in early phases of antiphosholipid syndrome. There is also a close relationship between oxidative stress and autoimmune diseases [135]. MDA is increased and sulfhydryl groups were decreased in patients with systemic lupus erythematosus [136]. PON-1 activity and vitamin E was lower in patients with other autoimmune diseases such as psoriasis, vitiligo, and alopecia [135]. Oxidative stress is associated with autoimmune thyroid destruction and N-acetyl-L-cysteine reduces ROS and restores thyroid morphology [137].

Oxidative Stress and Endocrinological Abnormalities

Five percent of women with unexplained infertility have elevated levels of FSH in the early follicular phase which suggests diminished ovarian reserve [138, 139]. FSH and LH abnormalities may reflect a dysfunction in the pituitary-ovarian axis [140]. Furthermore, serum estradiol levels in the follicular phase and the estradiol/progesterone ratio have been shown to be elevated in that group [138, 141]. An absent midcycle elevation of prolactin has been demonstrated in women with unexplained infertility [142]. The luteal phase was impaired in 30% of women with the presence of shorter luteal phase or decreased peak serum estradiol [138, 141]. Abnormal follicular LH pulse frequency or decreased midfollicular FSH level have been reported to induce an impaired luteal phase which may be related to functional imbalance in the hypothalamus [143]. Decreased inhibin-B and increased FSH concentrations may reflect a poor ovarian reserve [138, 144]. AMH and AMHRII polymorphisms were also associated with unexplained infertility [145].

Oxidative stress may have a close relationship with these endocrinologic abnormalities. It has been demonstrated that there is a positive association between oxidative stress and high FSH levels [146]. Exposure to supraphysiological levels of ROS is detrimental to oogonia [147]. ROS may contribute to mitochondrial dysfunction, low production of ATP due to impaired oxidative phosphorylation and impaired oogenesis, low oocyte number, and result in increased FSH and LH levels. LH surge is maintained by the oxidant/antioxidant balance [40]. Antioxidants have a positive effect in dominant follicle selection and also modulate the daily rhythm of LH and prolactin secretion and excess ROS may have a detrimental effect on these actions [32, 148]. Serum lipoperoxide levels were increased in women with luteal phase defects [149]. Antioxidants prevent luteal phase defects, they maintain progesterone production and protect corpus luteum against regression [33]. Another study demonstrated that elevated serum levels of advanced glycosylated end products (AGEs), markers of oxidative stress, have been positively correlated with serum AMH levels that may be another etiologic factor in unexplained infertility [150].

Oxidative Stress and Unexplained Infertility

Oxidative stress has been implicated in the pathophysiology of the disease. Lipid peroxidation marker, MDA, was increased and TAC was decreased in the peritoneal fluid of women with unexplained infertility [151]. It has been hypothesized that peritoneal fluid diffuses into the fallopian tube where it may cause damage to sperm.

Folate is a B9 vitamin that plays a role in amino acid metabolism, and the methylation of proteins, lipids, and nucleic acids. Acquired or hereditary folate deficiency results in homocysteine accumulation. Polymorphisms in folate-metabolizing pathways of genes may be responsible for unexplained infertility in this group [152]. Hyperhomocysteinemia may activate apoptosis leading to follicular atresia [153]. Pregnancy and implantation rates were lower and abortion rates were higher in women elevated homocysteine [154]. It disturbs the endometrium and contributes to poor oocyte quality [152]. eNOS mRNA in the endometrium of the women with unexplained infertility was significantly higher compared with controls [155]. Its increased expression suggests the detrimental effect of NO in endometrial receptivity and

implantation [155]. Excess NO could impair implantation through several mechanisms. NO has been found to induce endometrial epithelial apoptosis [156, 157]. Increased eNOS expression at luminal surface could induce epithelial apoptosis and implantation failure. The second mechanism is that NO may impair implantation through localized nitrosative stress. It is suggested that excess eNOS expression can create local oxidative stress which could impair implantation [11, 158].

Increased ROS in patients with unexplained infertility suggest reduced levels of antioxidants such as vitamin E and GSH would reduce ROS scavenging ability and prevent the neutralization of toxic ROS effects [159]; however, the use of antioxidants in women with unexplained infertility is unclear. Studies are needed to explore the efficacy of antioxidant therapy in these patients.

Conclusion

Oxidative stress is an imbalance between ROS and antioxidants. There seems to be a close relationship between oxidative stress and the underlying etiologic factors that may contribute to unexplained infertility such as defective endometrial receptivity, impaired oocyte quality, premature ovarian failure, minimal and mild endometriosis, tubal disease, pelvic adhesions, and immunological and endocrinological abnormalities. Moreover, it has been demonstrated that ROS can also negatively affect ovulation, fertilization, implantation, embryo quality, and pregnancy rates. Although oxidative stress may contribute to unexplained infertility, the role of antioxidant therapy in that group remains unclear. Further studies are needed to show the effectiveness of antioxidants in that group.

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