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The Role of Oxidative Stress in Spontaneous Abortion and Recurrent Pregnancy Loss: A Systematic Review

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Human reproduction is not considered a highly efficient biological process. Before the end of the first trimester, 30%–50% of conceptions end in spontaneous abortion. Most losses occur at the time of implantation. 15%–20% of clinical pregnancies end in spontaneous abortions. Recurrent pregnancy loss is a frustrating clinical problem both for clinicians and patients. Recurrent pregnancy loss affects 0.5%–3% of women in the reproductive age group, and between 50%–60% of recurrent pregnancy losses are idiopathic. Oxidative stress-induced damage has been hypothesized to play a role in spontaneous abortion, idiopathic recurrent pregnancy loss, hydatidiform mole, defective embryogenesis, and drug-induced teratogenicity. Some studies implicate systemic and placental oxidative stress in the pathophysiology of abortion and recurrent pregnancy loss. Oxidant-induced endothelial damage, impaired placental vascularization and immune malfunction have all been proposed to play a role in the pathophysiology of idiopathic recurrent pregnancy loss. Oxidative stress-induced placental dysfunction may be a common cause of the multifactorial and polygenic etiologies of abortion, recurrent pregnancy loss, defective embryogenesis, hydatidiform mole, and drug-induced teratogenic effects. Oxidative stress-induced modification of phospholipids has been linked to the formation of antiphospholipid antibodies in the antiphospholipid syndrome. The objective of this review was to examine the association between oxidative stress, spontaneous abortion and recurrent pregnancy loss, based on the published literature. We conducted an extensive literature search utilizing the databases of Medline, CINAHL, and Cochrane from 1986 to 2005. The following keywords were used: oxidative stress, abortion, recurrent pregnancy loss, reactive oxygen species, antioxidants, fetal development, and embryopathies. We conducted an electronic search, as well as a manual search of cross-references. We have included all studies in the English language found in the literature focusing on oxidative stress and its association with abortions, recurrent pregnancy loss and drug-induced teratogenicity. The role of antioxidant vitamins for primary prevention of oxidative stress-induced pathologies needs to be investigated further.

Target Audience: Obstetricians & Gynecologists, Family Physicians

Learning Objectives: After completion of this article, the reader should be able to state that the causes of spontaneous and recurrent abortion are multifaceted, however, some of the causes may be preventable and also explain that the role of oxidative stress during pregnancy and adverse pregnancy outcomes has a basis in pathophysiology, although the role of oxidative stress and the treatment of oxidative stress during or before pregnancy remains speculative.

By definition, spontaneous abortion is the termination of pregnancy before 20 completed gestational weeks from the last menstrual period, or less than 500 g (1) fetal weight.

The rate of spontaneous abortion in North American women is about 15%–20% and increases with age, from 15% in women younger than 25 years and to 35% in women older than 38 years (2). The rate of spontaneous abortion among women treated with clomiphene for infertility and human menopausal gonadotropin is about 25% (3). Wilcox et al conducted a prospective study of 221 women who were trying to conceive (4). The rate of spontaneous abortion was 12% among clinically diagnosed pregnancies. However, when biochemical pregnancies (those diagnosed by β-hCG assay) were taken into account, the rate of spontaneous abortion was as high as 31%. Another study also demonstrated a 12% rate of spontaneous abortion among clinically diagnosed pregnancies.

Recurrent pregnancy loss is highly frustrating for both patients and physicians. Classically, recurrent pregnancy loss is defined as 3 or more consecutive pregnancy losses before 20 weeks of gestation. The incidence of spontaneous abortion increases after miscarriage, from 13% in those with no previous miscarriage to 23% after 1 miscarriage, to 29% after 2 miscarriages, and to 33% after 4 miscarriages. Although chromosomal abnormalities are the cause of about 50% of all spontaneous abortions, the remaining 50% of the causes may be preventable (5). However, Bick et al reported a higher prevalence of coagulation and immunological alterations among patients with recurrent pregnancy loss. Still, the precise nature of the factors involved in the etiology of spontaneous abortion and recurrent pregnancy loss remains unclear.

ROLE OF OXIDATIVE STRESS

Although oxygen is essential for sustaining life in cells, it undergoes extensive metabolism that can result in the production of toxic derivatives. This metabolism is mainly confined to the electron transport chain in the mitochondria that ultimately results in the generation of ATP, which supports cell metabolism. The end products of oxygen metabolism may include molecules in an activated electronic state that have unpaired electrons and are highly reactive with molecules found in biological systems. Collectively, these activated molecular species derived from oxygen metabolism are designated as reactive oxygen species (ROS) (6). ROS extensively damage cellular organelles including the mitochondria, nuclear and mitochondrial DNA, and cell membrane, ultimately leading to cellular demise (7–9). However, ROS play a pathological role not only in cell function but also a physiologic one when present at very low levels. To prevent ROS-induced damage, cells have evolved antioxidant systems. As a result, there is a delicate balance between ROS production and antioxidant activity that maintains a physiologic balance leading to cellular homeostasis. When this balance is perturbed by an excess of ROS production, a state of oxidative stress ensues leading to cell damage and cell dysfunction (10,11).

The etiology of recurrent pregnancy loss includes chromosomal abnormalities, uterine anatomic anomalies, immunologic disorders such as antiphospholipid antibody syndrome, clotting disorders such as hyperhomocystinemia, and sperm DNA fragmentation (5). Oxidative stress has also been implicated as an important cause of recurrent pregnancy loss. Loss of antioxidant defenses have been shown to be associated with recurrent pregnancy loss (12). Biochemical markers of ROS-induced membrane damage such as lipid peroxidation products, reach high levels immediately before abortion (13). It has been proposed that an oxidant/antioxidant imbalance is associated with pregnancy loss (14).

FREE RADICALS

Under normal conditions, paired electrons create stable bonds in molecules found in biological systems. However, if the bond is weak, it may break, leaving unpaired electrons in the outer shell of the atom leading to the formation of free radicals which react to regain stability (15). This in turn leads to binding of the free radicals with one another, which initiates a cascade of reactions leading to uncontrolled chain reactions and generation of free radicals. The oxygen molecule, due to its extensive metabolism throughout the body, undergoes similar chain reactions that lead to the formation of oxygen free radi-

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cals ($O^{\cdot -}$). Oxygen free radicals may be produced normally, as a part of cellular metabolism or as a requirement for body defense. Relentless formation of oxygen radicals in the absence of proper antioxidant balance produces pathological changes in cells (Fig. 1). However, as indicated previously, free radical generation under controlled conditions plays an important role in cellular homeostasis. Increased oxidant formation and even the presence of oxidative stress is considered normal during the second trimester of pregnancy (16,17). Elevated generation of free radicals by placental mitochondria has been reported in pregnancy (18). Fait et al demonstrated increased generation of superoxide free radicals by polymorphonuclear leukocytes from pregnant women in their first trimester of pregnancy. In a prospective study, blood samples were drawn throughout pregnancy. The lipid peroxide levels remained the same and the vitamin E levels increased with increasing gestational age in women with uncomplicated pregnancies.

Free radicals can be classified as reactive oxygen species or reactive nitrogen species. ROS are mainly comprised of superoxide radicals, hydrogen peroxide (H_2O_2), singlet oxygen ($O^{\cdot -}$), and the hydroxyl radical ($OH^{\cdot -}$). The reactive nitrogen species are mainly comprised of peroxynitrites and nitric oxide, which may be generated during hypoxia and cause reperfusion injury in cells (19). Aside from the deleterious effects of free radicals on cells, many studies have shown their beneficial role in female reproduction. This mainly relates to follicular development (20), follicular atresia, ovulation, maintenance of pregnancy and implantation (21,22).

ANTIOXIDANTS

Because production of free radicals is the inevitable result of the aerobic metabolism of cells, counteracting their toxic effects with antioxidant defenses is part of the cell's metabolic strategy (Fig. 1). Antioxidants can be defined as "any substance that, when present at concentrations lower than an oxidizable substrate, significantly delays or prevents oxidation of that substrate" (23). These agents can exist in enzymatic and nonenzymatic forms. Common enzymatic defenses include superoxide dismutase (SOD), catalase, and glutathione peroxidase and glutathione reductase. Nonenzymatic agents like ferritin, ceruloplasmin, transferrin, ascorbic acid (vitamin C), and α-tocopherol (vitamin E) can also serve as antioxidants. 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

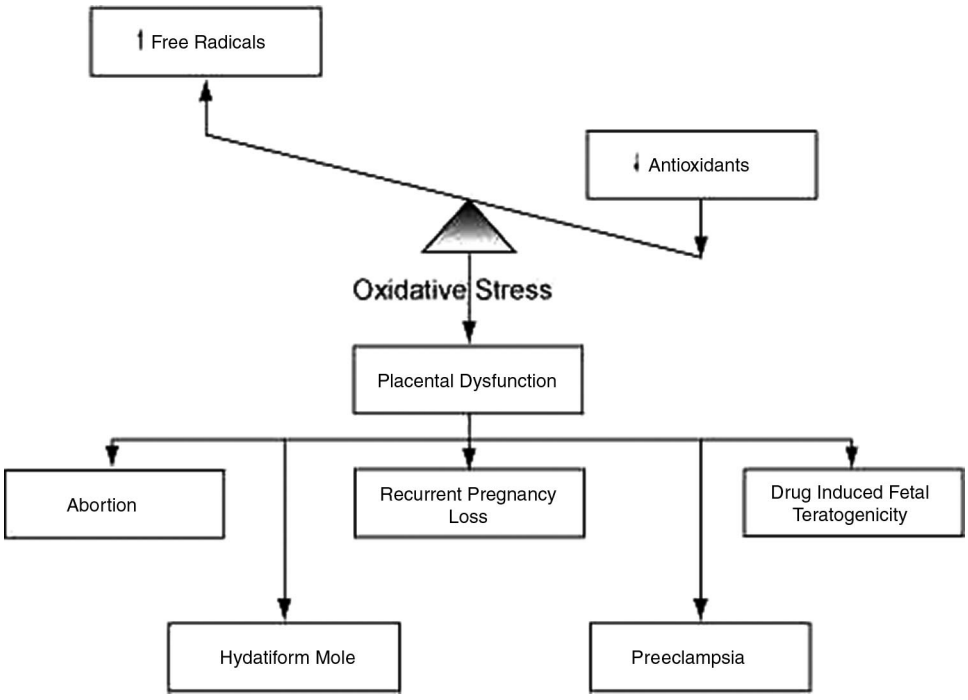

Fig. 1. Role of oxidative stress in recurrent pregnancy loss.

mitochondria, whereas Cu-Zn-SOD is mainly localized in the cytoplasm. Since ROS are highly reactive, it is essential that antioxidants be near the site of ROS production, so they can be quickly activated to be effective in preventing ROS-induced damage (24). The developing embryo can generate both intracellular and extra-cellular ROS, thus requiring effective antioxidant activity. Enzymatic antioxidant defenses have been documented in mammalian embryos and oocytes (25,26), and nonenzymatic defenses in tubal (26) and follicular fluids (27). Increased levels of antioxidants have been documented in normal pregnancy (28–30), whereas loss of antioxidant defenses have been observed in patients with recurrent abortion as a result of their increased consumption (12,31). The lower antioxidant levels could aggravate pro-oxidant injury on endothelial cells, altering prostacyclin–thromboxane balance and culminating in preeclampsia or abortion (17). The impact of the glutathione/glutathione transferase system has been particularly studied with regard to the occurrence of abortion, without excluding associated genetic polymorphisms (32, 33). Animal studies have demonstrated the protective effects of glutathione peroxidase against oxidative damage in neurogenic tissue development in fetuses (34). The glutathione peroxidase/reductase antioxidant system is a well-known scavenger of ROS, preventing lipid peroxidation in cells. Diabetes is a gluco-oxidative state, and leads to increased oxidative stress when associated with pregnancy. Increased oxidative stress may alter placental vasculature, leading to early miscarriages. Patients with diabetes and spontaneous abortion show an increase in glutathione peroxidase expression but reduced selenium levels, creating an intimate link between weak antioxidant defenses and early fetal loss (35).

Reduced glutathione not only acts as a free radical scavenger but also maintains the functional reserve of sulfhydril groups (SH)-containing antioxidants in the placenta. SH-containing antioxidants play a significant role in the onset of preterm labor, which is defined as initiation of labor at less than 37 weeks of gestation. A prospective study conducted in patients with preterm labor demonstrated an increase in glutathione peroxidase and glutathione reductase levels in the placenta. These levels were compared with those found in normal patients with no history of preterm labor. A statistically significant increase in this antioxidant system was found, suggesting the presence of a state of increased demand to oppose the effects of oxidative stress in such patients (36).

Nonenzymatic antioxidant deficiency has also been implicated in recurrent pregnancy loss. A direct relationship between selenium deficiency and abortion has not been established to date. Although a deficiency state has been found in various tissues in patients undergoing abortion, the exact mechanism has not yet been identified (37). A group of investigators explored the role of selenium deprivation and supplementation and recommended the performance of larger trials to establish the effects. The group stressed that previous studies that dealt with selenium levels in pregnancy showed discrepancies due to measurements in plasma. Thus a pilot study was conducted to estimate selenium levels in red blood cells in 20 women with 3 or more unexplained recurrent pregnancy losses. A statistically significant decrease was found in these patients (38).

The antiphospholipid (aPL) antibody syndrome is one of the known autoimmune causes of recurrent pregnancy loss. The syndrome is characterized by high titers of aPl or lupus anticoagulant associated with pregnancy loss or history of a thrombotic event and/or autoimmune thrombocytopenia. In a series of women testing positive for antiphospholipid antibodies, an 84% pregnancy loss rate was found, and 50% of these were fetal deaths (39). The pathophysiology of antiphospholipid antibody formation is not clearly defined; oxidative stress has been proposed to have a role in the formation of these antibodies. Increased oxidation of low density lipoproteins (LDL) alters the antigenic properties of some modified phospholipids and the aPL antibodies are directed against the oxidized phospholipids (40). Patients with preeclampsia, a condition associated with oxidative stress, also have been reported to have modified phospholipids. Increased oxidative stress in patients with preeclampsia also resulted in an increased rate of oxidation of phospholipids with increased titers of auto-antibodies to malondialdehyde-low density lipoprotein (MDA-LDL). Reports have linked the formation of anticardiolipin antibodies to the oxidative modification of phospholipids (40,41), and the cardiolipin molecule is very susceptible to oxidative modification; there is increased lipid peroxidation in patients with this syndrome (42). Oxidative stress has also been proposed to be the link between antiphospholipid antibodies and thrombosis and other vascular complications.

PLACENTAL OXIDATIVE STRESS

Normal human placentation is determined for the most part by the proper invasion of the uterine spiral

arteries by a genomically normal trophoblast. This invasion governs the changes in the anatomy of the placental vasculature to ensure optimum perfusion by the maternal vessels. Definite metabolic changes occur in embryos during the transition from first to second trimester. It is evident that during the period of embryonic organogenesis the prevailing oxygen tension is low and metabolism is largely anaerobic (43). Thus, the production of ROS perhaps is reduced to prevent DNA damage induced by oxidants. This is also supported by animal research indicating increased blastocyst development rate at low oxygen tension (44). At the end of the first trimester, a definite rise in oxygen tension occurs in the intervillous space from less than 20 mm Hg to >50 mm Hg (45, 46), leading to a burst in oxidative stress. Lower oxygen tension in the first trimester stimulates the invasive capacity of the trophoblast (47). This is probably due to increased activity of integrins that help trophoblast cells to proliferate. Persistent low oxygen tension also diminishes placental proliferation and invasion, and hence, increased oxygen tension enables persistence of cytotrophoblast proliferation (48). It is suggested that impaired placental development or degeneration of syncytiotrophoblast in early pregnancy may be an effect of placental oxidative stress that may lead to complications such as recurrent abortions, preeclampsia, and congenital anomalies in diabetes (49). Several biomarkers have been associated with preeclampsia and increased oxidative stress, and some of the primary culprits are NOS-1, an isoform of NADPH oxidase (50), and endothelin 1 (ET-1) (51). It is possible that some of these factors may play an inhibitory role in cell proliferation and maturation and trigger oxidative stress in the human placenta by altering the balance between oxidant (increased MDA levels) and antioxidants (decreased GSH, GSSG, and AA). This can result in cell apoptosis leading to derangements in placental invasion and early abortion.

Jauniaux et al investigated the placental circulation using immunohistochemical analysis for heat shock protein (HSP) 70i, a marker for cellular stress such as nitrotyrosine residues (N-Tyr) and hydroxynonenal (HNE) as markers of protein and lipid oxidative damage, respectively (52). In this case–control study in normal pregnancies, intervillous blood flow increased with gestational age, being detected in 9 of 25 cases at 8 to 9 weeks but in 18 of 20 at 12 to 13 weeks. In abnormal pregnancies, flow was detected in nearly all cases (22 of 25) at 8 to 9 weeks. Early flow was restricted to the peripheral regions of most normal placentas, whereas in missed miscarriage it was most common in central regions or throughout the placenta. Immunoreactivity of HSP 70i and N-Tyr was greater in samples from peripheral than from central regions of normal placentas, and from missed miscarriage compared to controls. The authors conclude that the oxidative damage to the trophoblast, induced by premature and widespread onset of the maternal placental circulation secondary to shallow trophoblast invasion, is a key factor in early pregnancy loss. High oxygen concentrations in the periphery of normal early placentas may similarly induce local regression of the villi, leading to formation of the chorion leve.

Placental morphology, immunohistochemical analysis for heat shock protein (HSP 70i) and markers of cellular stress such as nitrotyrosine residues (N-Tyr) and hydroxynonenal (HNE), which are markers of protein and lipid oxidative damage, respectively, were investigated in a case–control study (53). Cases were women undergoing evacuation for missed abortion and controls were women undergoing elective termination of pregnancy. Assessment of the percentage of the villous surface covered by healthy unstressed syncytiotrophoblast was less in missed miscarriage than in controls. In those areas where the syncytium was observed to be sloughing away, the degenerate layer stained intensely for the markers of oxidative stress. Also, the underlying cytotrophoblast cells stained more intensely for catalase than did neighboring cytotrophoblast cells beneath an intact syncytium. The staining intensity of Hsp70, N-Tyr, catalase, Cu, Zn-SOD, and MnSOD was significantly higher in the pre-77 day miscarriage samples than in the age-matched controls. In the post-77 day samples, the only significant differences were increased N-Tyr and decreased HNE immunoreactivity in the miscarriage samples (53).

Hydatidiform mole is a known placental malformation causing early miscarriage. The pathogenesis of this disease, characterized by grape-like degeneration of the placenta and genotypic abnormalities, can also involve free radical-induced damage (Fig. 1). Patients with complete hydatidiform mole (CHM) have a decreased antioxidant response compared to controls, indicating increased DNA damage (11). This has been related to a lower total antioxidant potential/capacity in patients with CHM (54). This same group also postulated that the pathogenesis of preeclampsia and CHM may involve oxidative stress-induced damage, since both exhibit similar inflammatory states with increased levels of cytokines including TNF-α and interleukin-6 (55,56).

OXIDATIVE STRESS AND RECURRENT PREGNANCY LOSS

Recurrent pregnancy loss, defined as 3 or more consecutive pregnancy losses before 20 weeks of gestation, has been reported to affect 0.5%–3% of women in the reproductive age group (57). There is profound emotional and psychological stress associated with recurrent abortion. Women experience moderate to severe grief and have feelings of guilt or anxiety accompanying abortions (58). The families find it difficult to cope with the situation, and these women may require psychotherapeutic support at times. The causative factors associated with recurrent pregnancy loss can be varied and multiple. The factors reported in the literature are genetic abnormalities, uterine anomalies, autoimmune diseases such as systemic lupus erythematosus, or antiphospholipid syndrome, blood clotting disorders such as hyperhomocystinemia or other types of thrombophilias, infectious diseases, endocrinopathies, polycystic ovary syndrome, sperm DNA fragmentation, and sperm meiotic alterations (5,59). In about 50%–60% of recurrent pregnancy losses, a causative factor cannot be identified and are therefore classified as idiopathic. Endothelial damage, impaired placental vascularization, and immune malfunction have all been proposed to play a role in the pathophysiology of idiopathic recurrent pregnancy loss.

The human placenta is hemochorial. Successful pregnancy requires the development of an adequate uteroplacental circulation. Many studies have demonstrated that the maternal arterial circulation is established in the placenta by 10–12 weeks of gestation (45,60–62). Aberrant placentation appears to be involved in the pathophysiology of early pregnancy loss (49). Abnormal placentation leads to placental oxidative stress and syncytiotrophoblast dysfunction, and it has been proposed as a cause of early abortion.

Pregnancy has been characterized as an inflammatory state with the leukocytes showing changes similar to those seen in sepsis (63). Early pregnancy is characterized by a rise in the peripheral polymorphonuclear leukocyte counts. Increased generation of superoxide radicals from activated polymorphonuclear leukocytes has been demonstrated during early pregnancy (64). Increased generation of reactive oxygen species was demonstrated in leukocytes by significantly higher levels of granulocyte spontaneous chemiluminescence in the recurrent abortion patients compared to a control group of healthy women (65). There were significant differences in the level of oxygen radicals generated by the granulocytes during the oxidative burst induced by opsonized zymosan and following stimulation with *N*-formyl-methionyl-leucyl-phenylalanine. In contrast, there was a lower maximum response to chemotactic peptides in the habitual abortion group, indicating an oxidant/antioxidant imbalance in this group.

Elevated plasma levels of lipid peroxides and glutathione, as well as lower levels of vitamin E and β-carotene, were reported in patients with recurrent abortion (12). A significant elevation in plasma glutathione levels was observed in pregnant women with a history of recurrent pregnancy loss (32). Enhanced lipid peroxidation has also been hypothesized to be associated with the pathophysiology of recurrent pregnancy loss due to antiphospholipid syndrome. Antioxidant supplementation with vitamins C and E resulted in reduced levels of the anticardiolipin antibodies in patients with antiphospholipid syndrome (66).

A disruption of the balance between the prooxidant and antioxidant factors may occur in patients with recurrent abortions. An increase in the scavenging of oxygen radicals by antioxidants results in a decrease or depletion of cellular antioxidant levels. Decreased concentrations of plasma ascorbic acid, α-tocopherol, total thiols, and erythrocyte reduced glutathione (GSH) in patients with unexplained recurrent pregnancy loss or in patients with autoimmune or luteal phase insufficiency reflect an increase in oxidative stress (31). However a study by Nicotra et al demonstrated no significant differences in plasma levels of triglycerides, cholesterol, cholesterol esters, phospholipids, lipoperoxides, vitamin E, and erythrocyte glutathione peroxidase activity in women with recurrent abortion compared with controls (67).

Glutathione and glutathione peroxidase are antioxidants that neutralize free radicals and lipid peroxides to maintain intracellular homeostasis and redox balance. In a large case–control study, gene polymorphisms of enzymes of the glutathione family, glutathione *S*-transferase class (GSTM1) were studied. Elevated risk of recurrent pregnancy loss was found to be associated with the GSTM1 genotype null polymorphism in patients with recurrent pregnancy loss.

Homocysteine is a thiol-containing amino acid which is involved in the sulfurylation and methylation metabolic pathways and has been proposed to have proxidant effects. Plasma homocysteine levels normally fall during pregnancy. Disorders of homocysteine metabolism are associated with fetal neural tube defects, recurrent pregnancy loss, preeclampsia,

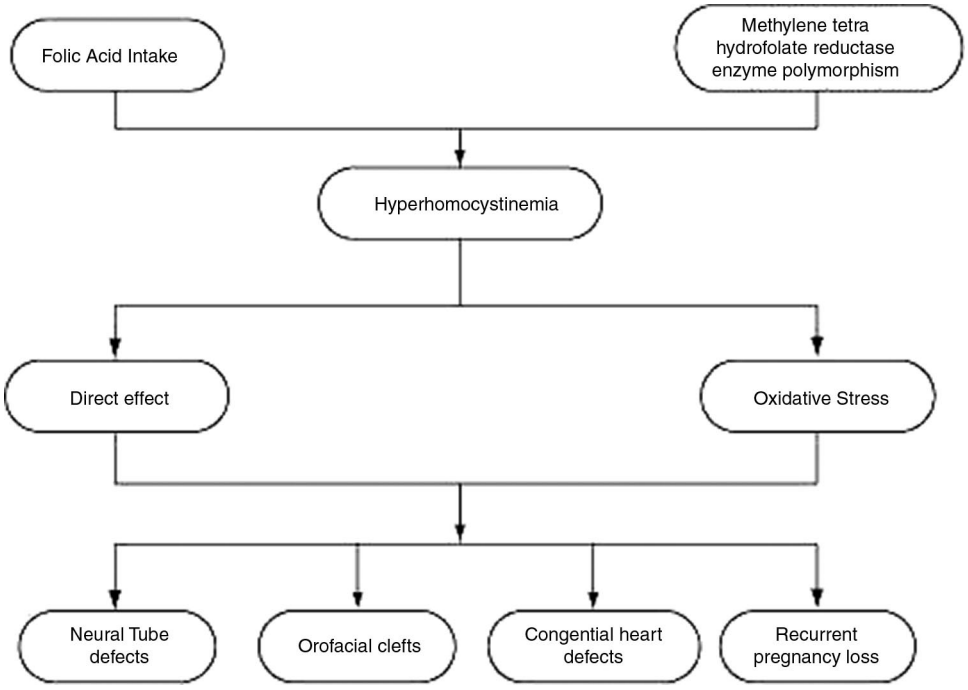

Fig. 2. Hyperhomocystinemia and pregnancy loss.

and placental abruption (Fig. 2). A recent meta-analysis estimated a higher pooled risk estimate for RPL with fasting and afterload hyperhomocystinemia (68). The meta-analysis concludes that hyperhomocystinemia is a risk factor for recurrent early pregnancy loss.

Selenium is a cofactor for the antioxidant enzyme glutathione peroxidase, which scavenges oxygen free radicals. Two of the 4 published studies investigating the association of selenium status with recurrent abortion indicated no significant differences in the levels between patients and controls (37,69). Two of the studies reported significantly lower levels of selenium in the recurrent abortion group (38,70). There is no conclusive evidence for selenium supplementation in recurrent abortion because of the drawback of the small sample size of the studies and the conflicting results.

SPERM DNA DAMAGE AND RECURRENT ABORTION

The paternal genome is of paramount importance in normal embryo and fetal development. ROS-induced sperm damage during sperm transport through the seminiferous tubules and epididymis is 1 of the most important mechanisms leading to sperm DNA damage (71–76). This results in single- and double-stranded DNA fragmentation (primary damage) and the generation of secondary DNA damage of the 8-OH-2′-deoxyguanosine type. Fertilization of the oocyte by a spermatozoon with unrepaired primary or secondary DNA damage may result in implantation failure, embryo development arrest, pregnancy loss, or birth defects (77–80). In addition, recent studies suggest that sperm DNA fragmentation may be associated with an increase in sperm aneuploidy (80,81). Sperm aneuploidy is mainly the result of meiotic alterations during spermatogenesis (82). ROS- and/or caspase- or endonuclease-induced DNA fragmentation may be increased in aneuploid sperm during passage through the epididymis (76). Therefore, couples diagnosed with recurrent pregnancy loss may benefit from testing of sperm DNA fragmentation in semen.

OXIDATIVE STRESS-INDUCED EMBRYOPATHIES

ROS-induced DNA damage has been implicated as a mechanism of drug-mediated teratogenicity (Fig. 3). When oxidative stress occurs, reactive oxygen species react with molecules in various biological systems, causing extensive cell damage and disruption of cell function. Various drugs such as phenytoin, thalidomide, and teratogens such as alcohol or cocaine, induce free radical-mediated damage to DNA, proteins, and lipids (83–85). Animal studies

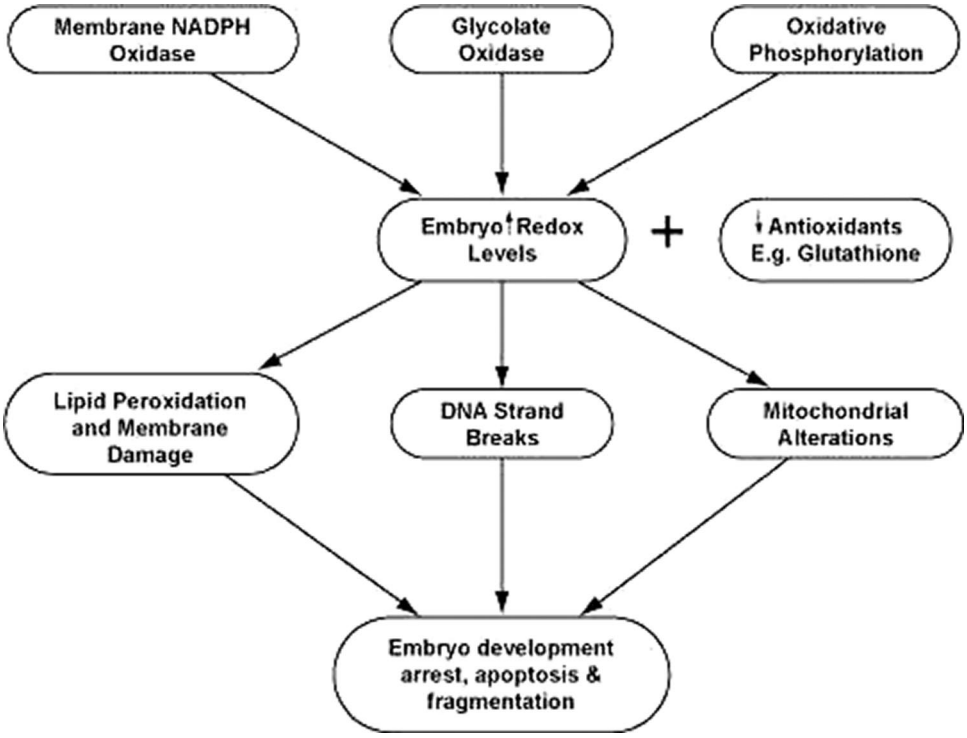

Fig. 3. Effects of oxidative stress on embryogenesis.

investigating the teratogenicity of drugs mediated through free radicals are still at the experimental stage. Homocysteine-induced oxidative stress has been proposed as a potential mechanism of apoptosis and disruption of palate development leading to cleft palate (86). Various endogenous antioxidant enzymes play a role in protecting the embryo against oxidative stress-induced DNA damage. Teratogenic drugs and xenobiotics can induce embryotoxicity through oxidative stress.

Inducible nitric oxide synthase (iNOS) is constitutively expressed in the conceptus. During organogenesis, iNOS, peroxynitrite, and oxygen radicals interact with each other, leading to the ROS-mediated teratogenic effects of xenobiotics. The teratogenic effects of phenytoin mediated through iNOS and peroxynitrite were demonstrated in a murine model (84). In a study conducted in rabbits, thalidomide was found to cause oxidative DNA damage. DNA damage has been implicated in the process of drug-induced teratogenesis. The thioredoxin/periredoxin group has been proposed as an active oxygen radical scavenging system (87). The significance of the role of thioredoxins was demonstrated in thioredoxin-deficient mice, which resulted in embryo death and smaller embryos with increased apoptosis.

The fetal alcohol syndrome has a high incidence in the United States. Heavy alcohol intake during pregnancy can result in fetal alcohol syndrome and its incidence is estimated to be high in the developed world. It has been proposed that antioxidant supplementation will overcome ethanol-induced ROS production and decrease fetal damage (88, 89). Ascorbic acid has been shown to inhibit alcohol mediated generation of ROS in embryos of *Xenopus laevis* and prevented microencephaly and intrauterine growth retardation in ethanol-exposed embryos (90). Optimal doses of antioxidants need to be determined for prevention of alcohol-induced fetal oxidative damage.

STRATEGIES AND INTERVENTIONS TO OVERCOME OXIDATIVE STRESS IN SPONTANEOUS ABORTION AND RECURRENT PREGNANCY LOSS

Although there is significant evidence suggesting that oxidative stress plays a role in the pathogenesis of spontaneous abortion and recurrent pregnancy loss, it is difficult to justify the use of antioxidant therapy in these patients. This is mainly due to the paucity of randomized studies. Although the litera-

ture suggests that antioxidant supplementation in in vitro fertilization has beneficial effects (90,91), very few studies demonstrate their role in preventing miscarriage. A meta-analysis suggests that vitamins play no role in preventing miscarriages (92), although they may reduce the incidence of preeclampsia. The lack of direct evidence is probably due to the omnipresence of oxygen in human tissues, as well as the limitation of human embryonic tissue for research (49).

Although oxidative stress plays a role in the pathogenesis of recurrent pregnancy loss, it is not the sole cause. As previously indicated, recurrent abortion may have multiple causes, including genetic, anatomic, autoimmune causes such as systemic lupus erythematosus, antiphospholipid syndrome, blood clotting disorders such as hyperhomocystinemia or other types of thrombophilias, infectious diseases, endocrinopathies, polycystic ovary syndrome, sperm DNA fragmentation, and sperm meiotic alterations (93). However, since increased oxidative damage to the placenta occurs in patients with recurrent pregnancy loss, supplementary antioxidant therapy may be of benefit to these patients during preconception and early stages of conception.

Vitamins are commonly prescribed during pregnancy. Although there is definite evidence that folic acid supplementation prevents neural tube defects, the role of vitamins in the prevention of spontaneous abortion and recurrent pregnancy loss has yet to be determined. It is well known that excessive alcohol intake during pregnancy can lead to abortion. Some studies demonstrated the beneficial effects of vitamins in reducing the effects of free radicals and liver malformations in rat fetuses induced by diabetes (94). Similar studies also demonstrated the effects of antioxidants in reversing the levels of γ-glutaryl transferase (GGT), a marker of alcohol-induced injury (95) and of glutathione reductase (96). Although the mechanism by which alcohol induces pregnancy loss may be both free radical-induced injury and other causes independent of oxidative stress, some experimental studies have shown encouraging results with antioxidant therapy (32,97,98).

Significant levels of antiphospholipid antibodies are reported to be associated with the syndrome of recurrent pregnancy loss, thrombotic events, and thrombocytopenic purpura. Several theories have been proposed to explain the formation of these ubiquitous antibodies to the phospholipids. Oxidative stress has been proposed to have a role in the pathophysiology of antiphospholipid (APL) syndrome associated with recurrent pregnancy loss. The enhanced formation of antiphospholipid antibodies has been linked to an increase in lipid peroxidation (42,99). Treatment of patients with this syndrome and recurrent pregnancy loss is challenging, and some interventions are not evidence-based. In a pilot study, antioxidant supplementation with vitamins C and E in patients with APL syndrome resulted in a significant reduction in the anticardiolipin antibody titers and an increase in the plasma levels of vitamins C and E (66). Several reports have suggested that treatment with antioxidants can reduce thrombin generation manifested by decreasing anticardiolipin antibody titers and lipid peroxidation-derived products (66). Administration of Probucol to patients with APL, a lipid-lowering agent with antioxidant properties, improved fibrinolysis, and normalized albumin excretion (100). Treatment of pregnancy losses associated with this syndrome remains complex. However, newer and targeted therapeutic agents such as cholesterol lowering agents with antioxidant properties need to be investigated.

The use of vitamins in pregnancy from the preconception period has been a subject of controversy. In fact, although it is now recognized that folic acid is effective in preventing neural tube defects, it was previously suggested that it increased orofacial defects and miscarriages (101) until these reports were challenged and disproved (102,103). Addition of vitamins C and E may strengthen antioxidant defenses in in vitro media (104) but human studies lack definite evidence. Evidence of increased teratogenicity associated with high dose vitamin A exists but normal vitamin consumption may not be harmful (105,106). In this respect, a recent meta-analysis is pertinent regarding the issue of vitamins and their role in preventing recurrent miscarriage. The objectives of this meta-analysis were to determine the effectiveness and safety of vitamin supplementation in women before conception, periconceptionally and in the early weeks of pregnancy, and their effects on spontaneous abortions, and maternal and fetal adverse outcomes. Pregnancies below 20 weeks and women of reproductive age group desirous of pregnancy were included. The study compared the effect of vitamins singly or in combination with other vitamins, placebo or no vitamins or other interventions to prevent miscarriages. The studies that were included were either randomized or quasirandomized trials. Tests for heterogeneity in the studies and causes of such heterogeneity were also determined. The meta-analysis ultimately included 17 trials and excluded 35. The vitamins that were supplemented included vitamin A alone or with folic acid, zinc, or multivitamins; vitamin C with or without multivita-

mins or vitamin E; folate with or without multivitamins and/or iron and multivitamins alone. In 15 trials, miscarriage or stillbirth as outcomes were reported. There was erratic reporting for miscarriages due to differences in defining criteria. As for the primary outcomes of total fetal loss, early or late, no difference was seen between women given any type of vitamin compared to controls. The trials for multivitamins demonstrated a lower rate of total fetal loss for women given multivitamins with or without vitamin A. Ultimately, the authors did not find any strong association between vitamin supplementation and reduction in incidence of early or late miscarriage, although the studies with multivitamin supplementation with or without vitamin A did have lower rates of total fetal loss. Positive primary outcomes regarding reduction of risk of preeclampsia with supplementation of vitamins C and E have been noted. Interestingly the group reported increased chances of multiple birth with intake of multivitamins with or without folic acid (92). Although the study rules out the beneficial effects of vitamins, it fails to demonstrate studies that show the increased rate of abortions due to lack of antioxidant defenses other than vitamins. More specific studies regarding type of vitamin and its preventive role need to be conducted.

CONCLUSIONS

Oxidative stress is present in most organs exposed to high oxygen metabolism such as the placenta. There is an emerging confluence of opinion that suggests that oxidative stress is one of the main underlying mechanisms in the pathogenesis of a continuum of disease processes such as spontaneous abortion, hydatidiform mole, and preeclampsia. Recurrent pregnancy loss may be caused by oxidative damage to macromolecules and DNA and ROS-induced signal transduction for various genes are some of the underlying factors leading to recurrent abortion. Oxidative stress and ROS-induced damage may be the missing pieces of the puzzle of abortion and recurrent pregnancy loss of unexplained etiology. The various causative factors of early abortion and recurrent miscarriage ultimately may lead to depletion of antioxidant defenses. Variations in antioxidant levels have been documented and related to miscarriage, but there is a lack of consensus, and therapy with antioxidants is yet to be universally accepted. Some of the ongoing antioxidant trials should provide answers on their safety and effects on maternal and fetal outcomes. Folic acid supplementation in the periconception period leads to significant reduction in the homocysteine levels which are conspicuous in women with hyperhomocystinemia and homozygous for MTHFR gene mutations. Lack of multivitamin supplementation with folic acid at the doses required periconceptionally will prevent neural tube defects and may have additional benefits in preventing preeclampsia. Antioxidant and vitamin supplementation at optimal doses may be especially beneficial in women with nutritional deficits. Trials conducted have been inherently poor because of bias introduced by lack of definite inclusion criteria, concealment, and loss of follow-up. Further well-designed and effectively monitored randomized control trials need to be conducted to document the safety and efficacy of vitamin and antioxidant supplementation to prevent pregnancy loss.

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