

Review Article

Role of reactive oxygen species in gynecologic diseases

RAKESH K. SHARMA and ASHOK AGARWAL*

Center for Advanced Research in Human Reproduction, Infertility, and Sexual Function, Glickman Urological Institute and Department of Obstetrics and Gynecology, The Cleveland Clinic Foundation, Cleveland, Ohio, USA

Free radicals derived from molecular oxygen and nitrogen are highly reactive metabolites called reactive oxygen species (ROS). Cells continuously produce free radicals and ROS as part of the metabolic process. They are involved in the various functions of the reproductive system. Antioxidants are enzymes or compounds that scavenge and reduce the presence of free radicals. Normally, a balance exists between concentrations of reactive oxygen species and antioxidant scavenging systems. The disruption of the delicate balance between pro- and antioxidants results in oxidative stress. Oxidative stress has been implicated in embryo fragmentation,

DNA damage, apoptosis and poor pregnancy outcome. It has also been implicated in a large number of gynecologic diseases, such as endometriosis, pre-eclampsia and maternal diabetes. The use of antioxidants may be beneficial in combating the harmful effects of oxidative stress in many of these diseases. The present review outlines the importance of these species in the pathology of various gynecologic diseases. (Reprod Med Biol 2004; 3: 177–199)

Key words: antioxidants, DNA damage, oxidative stress, pregnancy, reactive oxygen species.

INTRODUCTION

FREE RADICALS ARE involved in the physiology of reproduction. Free radicals may be defined as any molecule that has one or more unpaired electrons. Oxygen radicals, such as the superoxide anion ($O_2^{\cdot-}$), the hydroxyl radical (OH^{\cdot}), hydrogen peroxide and hypochlorite radical ($OHCl^{\cdot}$) and the peroxy radical (ROO^{\cdot}), comprise the highly reactive group of oxygen species called the reactive oxygen species (ROS).^{1–8} Also included in this group are the reactive nitrogen radicals, such as nitric oxide (NO^{\cdot}) and nitric dioxide (NO_2^{\cdot}) free radicals.

Oxygen toxicity is an inherent challenge to aerobic life. Antioxidant defense mechanisms include a variety of enzymes, the most important of which are superoxide dismutase (SOD), which produces hydrogen peroxide from superoxide radicals, and catalase and glutathione (GSH) related enzymes, which decompose hydrogen peroxide. In addition, there are hydrophilic

and hydrophilic low-molecular-weight antioxidants, such as vitamin C, uric acid, GSH and vitamin E, which act directly with various ROS (Table 1).⁹ Oxidative stress is caused by an imbalance between production of ROS and the antioxidant capacity.^{1,6,7,10–13}

While the controlled generation of ROS may have physiological functions as signaling molecules (second messenger) in many different cell types, their uncontrolled production is considered as an important factor in embryo toxicity and teratogenicity during prenatal life. Lipid peroxidation is a normal phenomenon that occurs continuously at low levels in all humans. Uncontrolled peroxidation can damage enzymes, lipids, proteins and cell membranes, and results in cell injury and death. During pregnancy, excess lipid peroxidation is associated with pre-eclampsia. Oxidative stress has been implicated in aging, diet, health, and in the etiology of pathological conditions, such as maternal diabetes, myocardial infarction, cataract or rheumatoid arthritis.^{14,15}

OXIDATIVE STRESS AND INFERTILITY

Production and deleterious effects of ROS on embryos

MANY INVESTIGATORS BELIEVE that the concentrations of free radicals also play a major role in the implantation of fertilized eggs. A low superoxide

*Correspondence: Dr Ashok Agarwal, Director, Center for Advanced Research in Human Reproduction, Infertility, and Sexual Function, Glickman Urological Institute and Department of Obstetrics and Gynecology, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Desk A19.1, Cleveland, OH 4419, USA. Email: agarwaa@ccf.org
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Table 1 Enzymatic and non-enzymatic antioxidants and their reproductive functions

Antioxidant	Localization	Remarks
Enzymatic antioxidants		
Superoxide dismutase	Cytoplasmic (Cu, Zn-SOD). Mitochondrial (Mn-SOD), endometrial glandular cells	Neutralizes intra- and extracellular superoxide anions. Promotes an increase in the proportion of zygotes undergoing at least one cleavage. Improves cleavage past the 2-cell block, increases blastocyst development. Decreased concentrations in pregnancies complicated by preclamsia. Elevated levels of Cu, Zn-SOD elicits a protective effect against diabetes-associated embryopathy.
Catalase	Tubal fluid	Neutralizes intra- and extracellular hydrogen peroxide, Promotes an increase in the proportion of zygotes undergoing at least one cleavage.
Glutathione peroxidase	Glandular epithelium in endometrium	Reduction of hydrogen peroxide and lipid peroxides and other peroxides to water or alcohol. Eliminates hydroxyl radical.
Nitric oxide synthase	Endometrial glandular cells and surface epithelia	Endothelial eNOS most marked in mid-secretory phase.
Non-enzymatic antioxidants		
Vitamin E	Ovary or follicular fluid	Major chain breaking antioxidant in membranes, directly neutralizes superoxide anion, hydrogen peroxide, and hydroxyl radical. Increases number of embryos developing to the expanded blastocysts. Increases viability of embryos exposed to heat shock. Increases survival of explanted rat conceptuses <i>in vitro</i> , Supplementation reduces the risk of ovulating aneuploid and diploid oocytes in aged female mice.
Vitamin C	Ovary	Chain breaking antioxidant, competitively protects the lipoproteins from peroxy radicals and recycles vitamin E. Diverse antioxidant function. Reduces presence of sulfahydryls. Inhibit embryo development at higher concentrations. Supplementation reduces the risk of ovulating aneuploid and diploid oocytes in aged female mice. Characteristic of infertility. Deficiency produces ovarian atrophy, extensive follicular artresia. Supplementation inhibits follicular apoptosis.
Glutathione	Tubal fluid, oocyte/embryo	Neutralizes superoxide anion, reduced glutathione metabolizes hydrogen peroxide and hydroxyl radical, Diverse antioxidant function. Improves the development of zygotes through the 2-cell block to the morula or blastocyst stage. Protects embryos against ROS. Improves bovine embryo production. Prevents oxygen-induced embryonic malformation. Depletion causes increase in H ₂ O ₂ concentration. Depletion in embryonic cells during the critical periods of organogenesis plays a role in hyperglycemia-induced embryopathy.
N-acetyl-L- cysteine		May act as a precursor of glutathione and thus facilitates its biogenesis. Inhibits upstream events leading to NF-kappaB activation. Inhibits phospholipids metabolism, proinflammatory cytokine release, and NF-kappaB DNA-binding in human fetal membranes.
Carotene		Chain breaking radical
Ascorbate	Follicular fluid	Blocks DNA damage and supports cytoplasmic maturation in porcine oocytes.

Table 1 Continued

Antioxidant	Localization	Remarks
Cysteamine	Follicular fluid	Precursor of hypotaurine, maintains redox status in oocytes. Maintains GSH content. Increases synchronous pronuclear formation. Improves normal embryo development.
EDTA		Chelating agent. Necessary to overcome the 2-cell block. Enhances <i>in vitro</i> embryonic development.
Taurine	Tubal fluid, follicular fluid	Chelating agent, neutralization of cytotoxic aldehydes, improves embryo development.
Hypotaurine	Tubal fluid, follicular fluid	Neutralizes hydroxyl radicals, precursor of taurine.
Transferrin	Tubal fluid	Metal chelation, necessary to overcome the 2-cell block.
Thioredoxin		Protects embryo against oxidative stress and embryopathic effects.
Dithiothreitol		Improves fertilization. Prevents age-associated decrease in oocyte viability.

Cu, Zn-SOD, Cu-Zn-superoxide dismutase; EDTA, ethylenediaminetetraacetic acid; Mn-SOD, manganese-superoxide dismutase; NOS, nitric oxide synthase; ROS, reactive oxygen species; SOD, superoxide dismutase.

environment is an important determinant in the development of fertilized eggs, and optimal concentrations of NO are necessary for the implantation of fertilized eggs into mice.^{16,17} Glutathione peroxidase (GPX) is involved in the elimination of the hydroxyl radicals, which is one of the most active free radicals and can damage intracellular DNA and the cell membrane.

Numerous endogenous and exogenous conditions can induce oxidative stress on the embryos.^{18,19} Various metabolic pathways and enzymes can produce endogenous ROS, including oxidative phosphorylation, β -nicotinamide adenine dinucleotide phosphate reduced (β -NADPH) oxidase and xanthine oxidase (XO).^{20–25} Several exogenous factors can contribute to ROS production by embryos, such as oxygen concentration, metallic cations, visible light, amine oxidases, and spermatozoa.^{26–32} ROS can alter most types of cellular molecules, such as lipids, proteins, DNA, mitochondrial alterations, embryo cell block, adenosine triphosphate depletion, apoptosis and fragmentation, and freeze-thaw survival.^{2,4,16,33–41} In addition, embryos may have different sensitivities to ROS at different stages, for example, bovine embryos at the 9–12 cell stage are more resistant to exogenous H_2O_2 compared with zygotes and blastocysts. These differences in sensitivities are related to variations in thresholds of the defense mechanism.⁴²

Antioxidants and embryo development

Embryo 2-cell block is associated with an oxidative burst, suggesting that the embryo may be particularly vulnerable to ROS at this stage. At this time, the

embryo *in vivo* is present in the oviduct, which provides various radical scavengers.^{43,44} Female reproductive tract fluid contains high concentrations of certain amino acids, suggesting that they may play a role in pre-implantation development.^{45–49}

Several defense mechanisms against ROS are present both in embryos and their surroundings. *In vivo* embryos are protected against oxidative stress by oxygen scavengers present in follicular and oviductal fluids. Oxidative stress can be avoided by preventing against ROS formation, interception by antioxidants and repair. Metal chelation is a major means of controlling lipid peroxidation and DNA fragmentation, and metal-binding proteins such as transferrin are important in controlling potential radical-generating reactions. Numerous non-enzymatic compounds, such as vitamin A, ascorbate and pyruvate have antioxidant function.^{42,43,50–55} Other sulfur compounds such as GSH, taurine, hypotaurine and cysteamine are equally important in protecting the embryos against ROS.^{56–70} Enzymatic defense mechanism protecting oocytes and embryos include SOD, catalase or GPX.^{44,58,67,71–73}

Repair systems in oxidative stress and apoptosis

Both embryos and spermatozoa possess repair mechanisms to counteract the toxic effects of ROS. Newly fertilized eggs are capable of repairing the damaged DNA of spermatozoa.⁷⁴ Both *in vitro* and *in vivo* repair of damaged spermatozoa or injected DNA is known to occur in oocytes.⁷⁵ In addition, the pronuclei of oxidative-damaged

embryos can be rescued partly by transferring into normal cytoplasm.⁷⁶ However, the ability of mitochondrial DNA to auto-repair leads to a progressive decline in mitochondrial function.⁷⁷

Oxidative stress must be controlled during *in vitro* culture to optimize *in vitro* embryo production. Pro-oxidant/antioxidant balance within the follicular microenvironment is implicated in the maturation of the oocytes.^{78,79} Both oocytes and embryos must be protected by reducing oxygen concentrations in the gaseous environment, by a radical buffering system produced by co-cultured cells, and/or by adding supplements to the culture medium.³ DNA damage beyond the capacity to repair will result in apoptosis and fragmentation of the early embryo or morbidity in later life.^{80,81} This may be one of the causes of fragmentation in human embryos seen in *in vitro* fertilization (IVF) programs.

Extensive evidence has demonstrated that cellular redox status modulates various aspects of cellular function. Glutathione (GSH) has been shown to modulate many important aspects of oogenesis, fertilization and development.^{82–85} Redox status of the cell is a key factor mediating the apoptotic pathway, and GSH presumably plays a critical role in mediating apoptosis by influencing the redox status.⁸⁶ Oxidative stress induces aneuploidy during oocyte development, and has been linked to maternal aging-associated infertility.^{82,83}

Normal cells balance the redox system and defense systems to protect against ROS. If such defenses fail or if there is excess ROS formation, oxidative stress and cell death may occur via necrosis or apoptosis. Necrosis typically arises from acute pathological stimuli, while apoptosis typically is associated with mild toxic stimulation over prolonged periods of time. Apoptosis plays an important role in development and differentiation.⁸⁷ Evidence suggests that some oocytes may undergo apoptosis or degenerate during development and aging.^{82,88} Oxidation of cellular sulfhydryl (SH) groups has been implicated in the induction of apoptosis and in the disturbance of the meiotic spindles of murine oocytes during aging.^{89,90} Altering thiol-redox status in zygotes and blastocysts may result in cell cycle arrest, apoptosis and/or cell death.

Fragmented embryos have limited development potential and rarely result in implantation.^{91,92} Apoptotic configurations in fragmented embryos have been reported at a stage prior to blastocyst formation.⁹³ Significantly higher concentrations of H₂O₂ and apoptosis have been reported in fragmented embryos compared to non-fragmented and unfertilized human oocytes, and embryos.⁹⁴ Although the implantation rate is not significantly different, the ability to develop to blasto-

cyst and the development of live fetuses is reduced in DNA damaged spermatozoa. Therefore, when there is a genetic damage, though some embryos may reach to the blastocyst stage, natural selection ensures that most of them would abort and not go to term.^{95–97}

DNA DAMAGE AND PREGNANCY OUTCOME

IN NATURAL CONCEPTION, sperm DNA status is an essential prerequisite to the achievement of a successful pregnancy.^{98,99} Several studies have attempted to establish a correlation between sperm DNA integrity, cleavage rates and embryo quality.^{100–102} DNA damage can occur as a result of defective sperm chromatin packaging, apoptosis and oxidative stress.^{1,6,7,11,13,81,103–107} Whether DNA-damaged spermatozoa can impair the process of embryo development remains unclear.

Damage to sperm DNA may be linked to an increase in early embryo death.¹⁰² However, a greater miscarriage rate was reported in intracytoplasmic sperm injection (ICSI), possibly reflecting the use of genomically compromised spermatozoa.¹⁰⁸ The number of embryos that develop to the blastocyst stage have been shown to negatively correlate with both the blastocyst number and quality in cases of male infertility.^{109–111} Zygotes resulting from IVF have a higher blastocyst development rate over ICSI.^{112,113} Sperm from infertile men have an elevated rate of DNA abnormalities. These abnormalities may lead to abnormal blastocyst development, failed implantations and spontaneous miscarriages.^{98,101,103,114–119} The deleterious consequences of fragmented paternal DNA become evident when the embryonic genome is activated. DNA damage has been correlated with pregnancy outcome both *in vivo* and *in vitro* (Table 2).^{38,81,116,118,120–122}

Using the sperm chromatin structure assay (SCSA), men with DNA fragmentation $\geq 30\%$ are at greater risk for low blastocyst rates and failure to initiate an ongoing pregnancy. In addition, the high DNA stainability ($\geq 15\%$) also is indicative of poor IVF fertilization rates. The extent of DNA damage can provide valuable prognostic information to the physicians counseling couples before IVF and ICSI.^{122–124}

OXIDATIVE STRESS IN OTHER TISSUES AND FLUIDS

Oviduct

GENETIC EXPRESSION OF antioxidant enzymes involved in the mechanisms protecting embryos

Table 2 Correlation of DNA damage and outcome of various assisted reproductive procedures

References	Study population	ART procedure	DNA assay	Pregnancy outcome
81	143	IVF	TUNEL	DNA fragmentation negatively correlated with fertilization and embryo cleavage rate.
102		IVF	Chromomycin A ₃	Lower rates.
125	150	ICSI	Nick translation	
125	150	ICSI	TUNEL	Fertilization rate negatively correlated with DNA fragmentation.
98	165		SCSA	
95			TUNEL	Embryonic development related to high pregnancy loss when DNA damage >8%.
116	24	ICSI	SCSA	DNA denaturation lower in men that initiated pregnancy. No pregnancies when DNA fragmentation >27%.
126	75	IVF		Negative correlation with proportion of oocytes fertilized.
		ICSI		
127	140	IVF	<i>In situ</i> nick translation	No association of DNA damage with fertilization rate.
			Chromomycin staining	Pregnant patients had significantly lower NT levels.
99	215		SCSA	Decline in fecundability as the percentage of cells with abnormal chromatin are >40%.
128	15	IVF	Comet	Head and tail DNA parameters useful in predicting embryo quality.
100	40	IVF	Comet	
101	60	IVF	Comet	In ICSI cycles DNA damage associated with impairment of post fertilization embryo cleavage.
121	119	IUI	TUNEL	No pregnancies if fragmentation >12%.
129	33	IUI	SCSA	Both fertilization and embryo development negatively correlated with DNA denaturation.
		IVF		
		ICSI		
120	50	IVF	TUNEL	No significant difference in fertilization and embryo quality in IVF and ICSI. In ICSI pregnancy resulted when DNA fragmentation <10%. No pregnancy when DNA fragmentation >20%.
	54	ICSI		
124	34	IVF	SCSA	No differences seen in SCSA parameters between patients who got pregnant to those who did not in either or conventional IVF.
		ICSI		
123	249	IVF	SCSA	IVF and ICSI fertilization rates comparable between high- and low – DFI groups. HDS ≥15% resulted in lower fertilization rates. Blastocyst rates or pregnancy outcome not affected by HDS. ICSI may be indicated in men with HDS ≥15%. Men with ≥30% DFI at greater risk for low blastocyst rates and failure to initiate an ongoing pregnancy.
		ICSI		
130	306	IUI	SCSA	Higher chances of pregnancy and delivery in IUI with DFI ≤27% and HDS ≤10%. No differences in outcome of IVF or ICSI when DFI ≤27%. ICSI significantly better than IVF when DFI ≥27%.
		IVF		
		ICSI		

ART, assisted reproductive techniques; DFI, DNA fragmentation index; HDS, high DNA stainability; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, *in vitro* fertilization; NT, *in situ* nick translation assay; SCSA, sperm chromatin structure assay; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

against ROS has been reported in human and mouse oviducts.⁸ Different expression profiles of transcripts encoding for gamma glutamylcysteine synthetase (GCSD), GPX, Cu-Zn-superoxide dismutase (Cu-Zn-SOD), manganese-superoxide dismutase (Mn-SOD) and catalase have been reported in the mouse model, whereas in the human, only transcripts encoding for GPX, Cu-Zn-SOD and catalase have been detected. Differences in the gene expression patterns of these antioxidant enzymes may reflect the variations in the ability of embryos to develop *in vivo* and *in vitro*.

Umbilical cord

Umbilical cord lipid peroxide concentrations reflect the extent of cell membrane damage by ROS. Persistent intrauterine asphyxia may result in ischemia to the organs, leading to permanent damage, especially to the brain. Oxygen free radicals activity in the neonate at birth and its relationship to umbilical cord acid-base status has been investigated in singleton deliveries. Lipoperoxides levels, cord blood pH and base excess were significantly related with singleton deliveries.¹³¹

In cases of uncomplicated labor followed by spontaneous vaginal delivery, significantly higher lipid peroxide concentrations were seen compared with those delivered following elective Caesarean section. Especially noted was an increase in the levels of malondialdehyde (MDA) (105%), while the hydroperoxide increase of 27% and base excess increase of 78% were seen.^{132,133} High levels of free oxygen radical activity in the fetus are a function of the labor process as are the changes in acid-base balance.

Follicles and follicular fluid

Ascorbic acid deficiency characteristically produces ovarian atrophy, along with extensive follicular atresia and premature resumption of meiosis.¹³⁴ Ascorbic acid can be depleted both by oxidant scavenging, as well as by cellular secretion.¹³⁵ In the preovulatory follicles, ascorbic acid is depleted by the presence of luteinizing hormone and by incubating the follicle with agents known to generate ROS. Isolation of the oocyte also results in ascorbic acid depletion.¹³³

Transferrin suppresses ROS generation and is important for the successful development of follicles. Transferrin messenger ribonucleic acid (mRNA) expression has been demonstrated in granulosa cells of the human and mouse ovary but not in the oocyte.¹³⁶ In the follicular fluid, transferrin levels have been found to be

highly correlated with those in the serum, suggesting that the small contribution made by its localized synthesis in the granulosa cell may be important for some as yet unknown mechanism in follicle maturation.

In women undergoing IVF, significantly higher levels of ROS have been reported in the follicular fluid of women who got pregnant compared with those who did not. Developmental competence of oocytes retrieved during IVF-embryo transfer procedure is not related to increased levels of oxidative stress in the follicular milieu they originate from.^{137,138} Both lipid peroxidation and total antioxidant capacity were positively correlated with the pregnancy rate. Follicular fluid ROS and lipid peroxidation levels might be potential markers for predicting success in IVF patients.^{138,139}

Amniotic fluid

The presence of ROS has been demonstrated in the amniotic fluid collected both at second and third trimester. Antioxidant capacity is reported to correlate with gestational age and estimated fetal weights or neonatal birthweights. However, ROS levels may not necessarily be influenced by gestational age, estimated fetal or neonatal weights.¹⁴⁰

Fetal membranes

The production of ROS, prostaglandins, proinflammatory cytokines and proteases has been implicated in the pathogenesis of term and preterm labor. NFkappaB activated pathways and subsequent phospholipid metabolism, pro-inflammatory cytokine release and N-acetylcysteine (NAC), through its ROS scavenging affect, inhibits protease activity in human fetal membranes.¹⁴¹

Preterm premature rupture of membranes (PPROM) results initially from ROS damage to collagen in the chorioamnion, leading to tear/rupture in the membrane as demonstrated by epidemiological and by *in-vitro* studies.¹⁴² In addition, clinical studies also show that chorioamnion and amniotic fluid samples, obtained from PPRM patients, exhibit excessive collagen degradation and antioxidants protect the chorioamnion from ROS damage.

OXIDATIVE STRESS AND SMOKING IN WOMEN

OVER 20% OF the women of reproductive age in Europe and the USA regularly smoke cigarettes.¹⁴³ Tobacco is a major source of exogenous pro-oxidants;

ROS and free radical generators are present in both its gas and particulate phase.¹⁴⁴ Active smoking affects the pro-oxidant/antioxidant balance inside the Graffian follicle in women undergoing ovulation induction for IVF. Cigarette smoking is associated with increased intensity of lipid peroxidation inside the mature ovarian follicle, which is accompanied by the depletion of local antioxidant scavengers.¹⁴⁵ There is a depletion of both enzymatic and non-enzymatic antioxidants in the follicular fluid of smokers.^{78,146} The decreased antioxidant capacity of follicular fluid in the smokers is, most likely, a secondary phenomenon caused by the utilization of antioxidants in defense reactions neutralizing ROS originating from, or induced by, the tobacco smoke constituents. A relatively high diploidy has been reported in unfertilized oocytes originating from smoking IVF-embryo transfer patients, suggesting a smoking-related meiotic immaturity of the oocytes.¹⁴⁷ An increased risk of trisomy has been observed in the offspring of mothers who smoke cigarettes.¹⁴⁸ A shift of the pro-oxidant/antioxidant balance inside the ovarian follicle towards oxidative stress may provide another possible explanation of impaired folliculogenesis in female smokers undergoing IVF-embryo transfer.

OXIDATIVE STRESS AND PREGNANCY

CONCENTRATIONS OF LIPID peroxides in the placenta are higher than in blood.¹⁴⁹ Higher levels of lipid peroxides are reported in blood in pregnant than in non-pregnant women. During gestation, elevated levels are seen in the second trimester and these taper off later in gestation, decreasing further after delivery.¹⁵⁰ Lipid peroxides are also produced in placenta, but their pattern of change over the course of pregnancy is unclear.¹⁵¹

Lipid peroxidation levels in placental tissue follow a different pattern over gestation than levels in blood.¹⁵² Both peroxidation and antioxidation reactions are enhanced during pregnancy.^{153–155} Maternal levels of prostacyclin, thromboxane, vitamin E and lipid peroxides were measured throughout the gestation period.¹⁵⁶

During uncomplicated pregnancy, ROS levels are elevated,¹⁵⁷ however, they are counterbalanced by an increased activity of antioxidants.¹⁵⁸ Maternal levels of vitamin E and lipid peroxides are both increased in pregnancy compared with non-pregnancy. The increase in the vitamin E levels may be a result of the physiological response to pregnancy, and there may be increased binding capacity of the blood for vitamin E.¹⁵⁵ Increased lipid peroxides during pregnancy may be related to the increase in spontaneous auto-oxidation of serum lipids.

Placenta lipid peroxides apparently contribute to maternal circulating levels because plasma lipid peroxide levels decrease abruptly after delivery.¹⁵⁸ The uterine contractile activity may generate ROS through the process of repetitive ischemia and reperfusion during labor in healthy women at term. Concentrations of vitamin C are significantly lower in maternal plasma, amniotic fluid and fetal plasma of vaginal delivery, indicating that water soluble vitamin C scavenges ROS in the aqueous phase and recycles lipid-soluble vitamin E to combat ROS-induced tissue damage.¹⁵⁹

MULTIVITAMIN SUPPLEMENTATION AND DNA DAMAGE DURING PREGNANCY

THE EFFECT OF multivitamin/mineral supplementation during pregnancy on plasma levels of antioxidants and sister chromatid exchange (SCE) rate – an indicator of damage to DNA has been examined.¹⁶⁰ A significant increase in SCE rates were reported at 34 weeks, whereas in the supplemental group, although the SCE rates did not decrease, they prevented the increase induced by ROS generated from enhanced steroid hormones in the last trimester, suggesting that multivitamin/mineral-supplementation during pregnancy may prevent DNA damage to the altered hormonal profile.¹⁶⁰

OXIDATIVE STRESS IN SPONTANEOUS ABORTION

SPONTANEOUS ABORTION IS accompanied by a profound disruption of the pro-oxidant-antioxidant homeostasis towards oxidative stress.¹⁶¹ Administration of antioxidants confers partial protection from the consequences of surgically induced oxidative burden.¹⁶²

OXIDATIVE STRESS IN GYNECOLOGIC DISEASES

Oxidative stress in the pathophysiology of endometriosis

ENDOMETRIOSIS IS A common gynecologic disorder that affects 8 to 10% of all reproductive age women, and is found in up to 50% of asymptomatic women undergoing laparoscopic tubal ligation.¹⁶³ It is characterized by the implantation and growth of endometrial tissue outside the uterine cavity. It is a multifactorial disease associated with a general inflammatory response in the peritoneal cavity. It is the leading cause of infertility.^{164,165} Pelvic pain or infertility caused by endometriosis

often cannot be distinguished on clinical grounds. The pathophysiology of endometriosis and the mechanisms responsible for its complication, in particular infertility, remain largely unexplained.¹⁶⁶ Involvement of various immunologic anomalies has been suggested in the past as a cause of these disorders.^{167–169}

Oxidative stress has been proposed as a potential factor involved in the pathophysiology of the disease. ROS may be involved in the endometrial-associated infertility and may play a role in the regulation of the expression of genes encoding immunoregulators, cytokines, and cell adhesions molecules implicated in the pathogenesis of endometriosis.¹⁷⁰ Increased production of hydroxyl radical and other free radicals in endometriosis is due to the activation of the immune system, and the various antigens in endometrial cells are excessively expressed.^{167,169,171–173} However, other studies based on direct measurement of ROS, NO, lipid peroxides and total antioxidant status have failed to demonstrate significant differences between the peritoneal fluid obtained from women with endometriosis compared to fertile women.^{174–178} The apparent discrepancy between these results may be due to the fact that only persistent markers of oxidative stress, such as enzymes or stable by-products of oxidative reaction may still be detected when endometriosis is diagnosed. Another possible explanation is that oxidative stress occurs only locally, for example at the site of bleeding, and does not result in an increase in total peritoneal fluid concentrations.

Increased presence of iron and lipid peroxidation

Retrograde menstruation is likely to carry highly pro-oxidant factors, such as heme and iron, into the peritoneal cavity, as well as apoptotic endometrial cells, which are well known inducers of oxidative stress. The role of increased iron levels in the peritoneal fluid of patients with endometriosis however, does not show altered levels of MDA, even though iron concentrations correlated with the severity of the disease. These results indicate that elevated levels of iron in these patients may not be involved in catalyzing free-radical reactions as judged by the degree of lipid peroxidation.¹⁷⁹

Nitric oxide and oxidative stress

The role of NO and oxidative stress in the pathogenesis of endometriosis-associated infertility has been examined. No increase in total antioxidant status (TAS) or the concentration of products of NO metabolism were

reported in women with endometriosis during the early follicular phase, suggesting that NO or TAS may not be the major cause in the pathology of endometriosis.¹⁷⁵ Persistently greater levels of endothelial NO synthase expression have been reported in endometriosis compared to controls throughout the menstrual cycle.^{168,180}

Glutathione peroxidase, superoxide dismutase and xanthine oxidase in endometrial tissues

Phase-dependent changes of GPX expression are seen in the surface and glandular epithelia in the eutopic endometrium during the menstrual cycle in the fertile control. However, this is lost in the eutopic endometrium in endometriosis. The aberrant expression of GPX in the eutopic endometrium throughout the cycle suggests a pathological role in endometriosis and adenomyosis.¹⁸¹ In endometriosis, expression of SOD is pronounced in the endometrium throughout the menstrual cycle, suggesting that superoxide plays a key role in infertility in endometriosis.¹⁶⁸ There is an exaggerated expression of Cu, Zn-SOD and Mn-SOD in eutopic endometrium in endometriosis and adenomyosis. The distribution of XO in eutopic and ectopic endometrium in endometriosis and adenomyosis has also been examined.¹⁷⁸

Oxidatively modified lipids in the peritoneal fluid

Evidence of oxidative stress in the peritoneal fluid of women with endometriosis and the presence of oxidatively modified lipids in the peritoneal fluid has been demonstrated.⁵ Oxidation-specific epitopes and macrophages are present in the endometrium and in endometriosis.¹⁸² Lipid peroxides interact with proteins, resulting in oxidatively modified proteins that are antigenic. Autoantibodies to oxidatively modified proteins have been detected in subjects with coronary artery disease, pre-eclamptic pregnancy and other vascular disorders.^{183,184} The detection of increased anti-oxidized modified low-density lipoproteins autoantibody titer is thought to represent a biologic marker of enhanced low-density lipoprotein oxidation *in vivo*.^{183,185}

A significant increase is seen in the autoantibodies to markers of oxidative stress were in women with surgically confirmed endometriosis, indicating that these women have enhanced oxidative stress.¹⁸⁶ It is not known whether inhibition of oxidation decreases the severity of the symptoms of endometriosis. Conversely,

if antioxidant treatment of endometriosis decreases the titer of the antibodies, this might indicate the source of oxidative stress.

Macrophages through scavenger receptor(s) play an important role in the uptake and removal of modified proteins, which can be formed as a result of inflammatory processes.¹⁸⁷ Scavenger receptors also known as oxidized low-density lipoprotein receptors do not recognize native low-density lipoprotein.¹⁸⁸ Oxidatively modified lipid-proteins constitute ligands for scavenger receptor(s). The presence of macrophages expressing scavenger receptor(s) in the peritoneal cavity of women with endometriosis may indicate oxidative damage to cellular or acellular components of the peritoneum.

Staining with antibodies to oxidatively modified lipid proteins (HNE-2 and MDA2) in both endometrium and endometriosis tissues containing stromal cells, in contrast to the controls, strongly indicates the occurrence of oxidative stress in endometriotic tissue. Although macrophages themselves are capable of inducing oxidation, a variety of other cells share this capacity. Ectopic endometrium may be a possible source of oxidized lipid proteins in the peritoneal cavity, also it can be speculated that endometriotic implants could promote a pro-oxidant environment in the peritoneal cavity of women with endometriosis.¹⁸²

Several hypotheses have been proposed to explain why oxidative stress is induced in cases of pelvic endometriosis. Erythrocytes, apoptotic endometrial tissues, and cell debris transplanted into peritoneal cavity by menstrual reflux and macrophages have been implicated as potential inducers of oxidative stress.^{173,183} Available data do not clearly indicate whether oxidative stress associated with pelvic endometriosis results from increased production of ROS, defective antioxidant defense or both conditions. Information is lacking on how the peritoneal environment, and particularly mesothelial cells, responds to the presence of blood, which is repeatedly carried into peritoneal cavity by retrograde menstruation, ovulation and bleeding lesions. The expression of antioxidant enzymes by endometrial cells appears to be enhanced in patients with developing endometriosis. The occurrence of oxidative stress suggests that despite this increase in antioxidant defenses, detoxifying mechanisms might be overloaded in endometriosis.

OXIDATIVE STRESS IN PRE-ECLAMPTIC PREGNANCY

PRE-ECLAMPSIA IS A leading cause of maternal and neonatal mortality and morbidity. It is a complex

syndrome of undetermined etiologic origin, usually diagnosed during the second half of pregnancy, with clinical features of hypertension, proteinuria and edema. It is characterized by endothelial cell dysfunction and lipid peroxidation, and alterations of immune responses may be involved in the pathogenesis.¹⁹⁰⁻¹⁹² Currently, there is no cure for pre-eclampsia except premature delivery.

Oxidative stress

There is increasing evidence that oxidative stress may be an important contributing factor to the pathogenesis of pre-eclampsia.¹⁹³⁻¹⁹⁸ Pregnant women affected by pre-eclampsia may have abnormal ROS production, particularly NO[•] and O₂^{•-}, abnormal levels of antioxidant defenses and increased placental lipid peroxidation.¹⁹⁹ In severe pre-eclampsia, the balance between ROS and antioxidants is disturbed due to an increase in oxidants and compromise of antioxidants.^{192,194-197}

Neutrophil activation and pre-eclampsia

Activated neutrophils have been implicated in the pathophysiology of pre-eclampsia and may play a significant role in the diverse manifestation of the disease, such as vascular endothelial damage and dysfunction associated with pre-eclampsia.^{204,205} Increased production of ROS is associated with neutrophil activation in pre-eclampsia. Neutrophils isolated from women with pre-eclampsia during the third trimester showed increased sensitivity to agonist stimulation and produce significantly more ROS than age-matched normotensive controls. This highlights the role of neutrophils in the oxidative stress and associated endothelial dysfunction that are characteristic of pre-eclampsia.²⁰⁶

Endothelium-derived nitric oxide

Data suggest that endothelium-derived NO may be increased in women with pre-eclampsia.²⁰⁷ An increase in XO activity has been proposed as a possible cause of oxidative stress in pre-eclampsia.²⁰⁸ Besides causing direct inactivation of NO[•] as a vasodilator, XO-derived O₂^{•-} reacts with NO[•] to form a potent peroxynitrite (ONOO⁻), which has been detected both within the vasculature and in the vessel walls of the placenta after pre-eclampsia.^{209,210} Increased endothelial NO synthase, decreased SOD, and increased nitrotyrosine staining in the maternal vasculature of women with pre-eclampsia have also been reported, indicating

increased peroxynitrite formation. Decreased bioavailability of endothelium-derived NO, due to oxidative destruction of NO by ROS, might contribute to the pathology of pre-eclampsia, a phenomenon in which antioxidant vitamins may play a beneficial role.

Endothelial cell dysfunction

Pre-eclampsia is characterized by endothelial cell dysfunction and lipid peroxidation. Activation of cell-mediated immunity caused by the alteration of immune response, especially caused by T-cell activation, may be involved in the pathogenesis of pre-eclampsia and release of endothelial ROS. Total antioxidants concentration has been shown to be negatively correlated with fibronectin, indicating endothelial cell damage and positively with creatinine clearance.²¹¹ These changes are early events in the development of pre-eclampsia, which may cause endothelial cell dysfunction in pre-eclampsia.

Another leading theory of the pathophysiology of pre-eclampsia is that oxidative stress induces vascular endothelial cell dysfunction.²¹² Advanced glycation end products (AGE) are circulatory molecules that can generate ROS and vascular dysfunction through an association with cell surface receptors (RAGE). Insulin resistance and obesity, as well as conditions that would increase RAGE levels, can induce pathophysiologic changes similar to those observed in women with pre-eclampsia.²¹²

Vascular-cellular adhesion molecule and inflammatory reactions

Pro-inflammatory cytokines, bacterial endotoxins, ROS and lipid peroxides can stimulate vascular cellular adhesion molecule-1 (VCAM-1) over expression on the surface of the cell. Increased concentrations of soluble VCAM-1 A as well as over expression of the percentage of CD49d⁺ peripheral blood and CD49d⁺ decidual lymphocytes have been reported in pre-eclamptic women.²¹³ Immunological mechanisms similar to inflammatory reaction may therefore be involved in pathogenesis of pre-eclampsia in peripheral blood as well as locally inside maternal-fetal interface.

Lipid peroxidation

The rate of lipid peroxidation in the placenta is abnormally high in the serum and the plasma of women with pre-eclampsia.^{214–216} As normal pregnancy advances,

the antioxidant activity of vitamin E and the vasodilating actions of prostacyclins are progressively favored with advancing gestation. However, this is not true in pathological pregnancies such as pre-eclampsia, where there is a reversal in these ratios to favor the vasoconstrictive actions of thromboxane and the toxic action of lipid peroxides.^{214,217} Both enhanced lipid peroxidation and alteration of immune responses are involved in endothelial cell dysfunction in pre-eclampsia.^{218–221}

The source of pre-eclampsia is unknown, but poorly perfused placental tissue may trigger free radical process and initiation of generalized lipid peroxidation.¹⁵² Increasing evidence supports the role of abnormal lipid metabolism and circulating modified lipids in the pathophysiologic mechanism of pre-eclampsia. In particular, lipids peroxides and blood oxidative imbalance are proposed as part of the cytotoxic mechanisms leading to endothelial cell injury.²²² Enhanced lipid peroxidation may result from excessive production of reactive oxygen species by neutrophil activation in pre-eclampsia.²¹¹ The increase in antioxidants is probably of a compensatory nature responding to the increased peroxide load in pre-eclampsia and may reflect the severity of the disease.

β -Nicotinamide adenine dinucleotide phosphate reduced oxidase and reactive oxygen species production

Pre-eclamptic women have circulating agonistic antibodies (AT1-AA) directed at the angiotension (Ang) II receptor (AT1).^{223,224} The effect of AT1-AA on ROS, NADPH oxidase expression and NFkappaB activation in women with pre-eclampsia has been studied.²²³

Genetic factors

Genetic factors may contribute to the release of ROS and consequently the pathophysiology of the disease. Increased neutrophil ROS may be an important in mediating the endothelial damage seen in pre-eclampsia. Superoxide can initiate lipid peroxidation, resulting in endothelial cell lysis.²²⁵ There is evidence in the literature to suggest that pre-eclamptic cells are 'primed' by some factors in the plasma, rendering them more sensitive to agonists stimulation with increased ROS production.²²⁶ Tumor necrosis factor- α , platelet-activating factor and syncytiotrophoblast microvesicles are among the factors that may contribute.^{227–229} Women with pre-eclampsia may be genetically predisposed to have an NADPH oxidase and therefore to increased oxidative stress.²³⁰

Using lymphoblasts from pre-eclamptic women, significant elevation in the agonist stimulated (phorbol ester) NADPH oxidase-mediated ROS production have been reported.²³¹ Enhancement of ROS generation might be important in mediating the endothelial dysfunction seen in pre-eclampsia. Tyrosine-dependent mechanisms may be implicated in control of the NADPH oxidase-associated increased ROS production with pre-eclampsia. Therefore, it is important to investigate the identification of low abundance-specific proteins that could be tyrosine phosphorylated, involvement of these proteins in the signal transduction pathways in pre-eclampsia, and their investigations as targets for pharmacological manipulation. There may be some underlying genetic predisposition. Oxidative stress has been suggested as a link between the two-stage model of the pre-eclampsia syndrome; maternal factors cause reduced placental perfusion (stage 1) and stage 2 involves activation of the maternal endothelium with multisystem disorders.¹⁹⁸

OXIDATIVE STRESS IN PREGNANCY INDUCED HYPERTENSION

HYPERTENSION COMPLICATES 6–20% of all pregnancies and ranks among the four most common causes of maternal/perinatal morbidity. Gestational hypertension is hypothesized to be due to pre-existing maternal disorders, such as obesity, insulin resistance, hypertension or renal disease.²³² All these different causes converge, resulting in pre-eclampsia by a common mechanism of endothelial cell activation and injury.²³³ Pregnancy-induced hypertension (PIH) is an endothelial dysfunction with an impaired effect of NO, the main regulator of the fetoplacental blood flow, which causes a maternal systemic vasodilatation during normal pregnancy.²³⁴

Oxidative stress has been demonstrated in the plasma samples of patients with hypertension by the elevated levels of uric acid and lipid peroxidation products. Activation of XO system may be a potential source of free radicals in PIH.²³⁵ Significantly lower levels of reduced GSH concentrations and increased levels of peroxides have been reported in patients with PIH compared with results in normal pregnancies. PIH has been associated with overproduction of lipid peroxides and impaired antioxidant defense.²³⁶

Both an increase in ROS and lipid peroxides, as well as a compromised antioxidant status, has been implicated in the pathophysiology of severe pre-eclampsia. Higher levels of antioxidant vitamins E and uric acid,

and lower levels of vitamin C have been reported in women with PIH and pre-eclampsia compared with normal pregnant and non-pregnant women.²³⁷

Lipid peroxidation has been suggested as a causative factor in PIH.^{202,238,239} In addition, low levels of plasma antioxidant activity and plasma levels of antioxidant vitamins (vitamins E and C) are seen in these women.^{200,207}

Pregnancy-induced hypertension complicates 5–10% of pregnancies, and a distinction must be made between pre-eclampsia and gestational hypertension, a usually more benign form of hypertension in pregnancy. Some classifications of pre-eclampsia require the presence of proteinuria, whereas others do not.^{240,241} The presence of proteinuria is a strong indicator of women at risk for perinatal complications and a poor pregnancy outcome. However, a small proportion of women with non-proteinuric gestational hypertension have similar perinatal results as patients with pre-eclampsia as defined by hypertension and proteinuria.²⁴²

Significantly increased levels of lipid peroxide, both in serum and placental tissue, and significantly decreased levels of vitamin E in serum, have been reported in women with severe gestational hypertension and pre-eclampsia compared with controls.²¹⁵ Similar values of lipid peroxides or vitamin E as controls have been reported in the group with mild gestational hypertension or chronic hypertension. Percent increase in lipid peroxide levels in women with severe gestational hypertension and pre-eclampsia appears to be higher in placental tissue than in serum. This correlates with the notion that the placenta acts as a source of lipid peroxides and that these changes are a primary event in the pathophysiology of endothelial lesions.²²² The severity of hypertension is inversely correlated with concentration of vitamin E.²¹⁷

Use of antioxidants in pre-eclampsia treatment

Supplementation with vitamins C and E may be beneficial in the prevention of pre-eclampsia in women at increased risk of the disease.²⁴³ The potential benefits of antioxidant supplementation on markers of endothelial and placental function, using vitamin C (1000 mg/day), vitamin E 400 IU/day) or placebo at 16–20 week's gestation, have been examined. In normal pregnancy, the ratio of plasma markers of endothelial activation – plasminogen-activator inhibitor 1 (PAI-1) and placental dysfunction (PAI-2) is decreased, but is high in pre-eclampsia owing to endothelial-cell activation and

placental insufficiency. Vitamin supplementation was associated with a 21% decrease in PAI-1/PA II ratio during gestation.²⁴³ Larger multicenter trials of high-risk women from different populations are needed to further validate the benefits of multivitamin supplementation. This could be a relevant target for future clinical trials.

OXIDATIVE STRESS IN MATERNAL DIABETES

CONGENITAL ANOMALIES AMONG infants of diabetic mothers occur at a rate of 6–10%. This represents a two- to fivefold increase over that observed in the general population. These congenital malformations most commonly involve the central nervous system and the heart, and account for approximately 40% of the perinatal mortality and morbidity seen in pregnancies complicated by pregestational diabetes. Maternal diabetes leads to a higher frequency of congenital malformations in offspring compared with offspring of non-diabetic mothers.²⁴⁴ The teratological processes in diabetic pregnancy are not completely understood.²⁴⁵ There are excellent reviews on the role of free radicals in the etiology of diabetes, role of altered antioxidant defenses in the development of complications, role of oxidation of plasma lipids and lipoproteins in the development of atherosclerosis in diabetes and role of oxidative stress in the development of complications in diabetes.^{246–249}

Alterations in prostaglandin metabolism and role of cyclooxygenases

Diabetes-induced alterations of several metabolites, such as arachidonic acid and prostaglandins, in particular prostaglandins E₂ (PGE₂), have teratological capacity.^{250–254}

One of the mechanisms for diminished PGE₂ production in the embryo may be decreased cyclooxygenase (COX)-2 activity,^{255,256} this constitutes a possible link between the functional state of this enzyme and embryonic dysmorphogenesis. Inhibition of COX, a rate-limiting enzyme of prostaglandin biosynthesis causes developmental disturbances analogous to those seen in embryos exposed to high glucose concentrations.²⁵⁰ The addition of either arachidonic acid or PGE₂ to the culture medium with COX inhibitors in low glucose corrected the embryonic development, and PGE₂ supplementation also normalized the development of embryos cultured with COX inhibitors in high glucose concentration. Both SOD and NAC were able to diminish the dysmorphogenesis induced by the COX inhibitors, at doses previously shown to diminish

glucose-induced embryo damage in the same *in vitro* culture system. The addition of NAC normalized the morphology and 8-epi-PGF₂α concentration of the embryos exposed to high glucose without normalizing the COX-2 expression in embryos and membranes. The PGE concentration of day 10 embryos and membranes was reported to decrease after exposure to high glucose *in vitro* or diabetes *in vivo*. The addition of NAC *in vitro* to high glucose cultures largely rectified morphology and restored PGE₂ concentration.

Hyperglycemia/diabetes induced down regulation of embryonic COX-2 gene expression may be a primary event in diabetic embryopathy, leading to lowered PGE₂ levels and dysmorphogenesis. Antioxidant treatment does not prevent the decrease of COX-2 mRNA levels but restores the PGE₂ concentrations, indicating that diabetes-induced oxidative stress aggravates the loss of COX-2 activity, thereby partly explaining the antiteratogenic affect of antioxidant treatment.

Role of reactive oxygen species in the induction of dysmorphogenesis

The direct demonstration of ROS production has been controversial, in both short-term incubations of embryonic cells and *in vivo* measurements.^{257–259} A putative excess of ROS has been observed in studies during which diabetes-induced embryopathy was blocked by antioxidants *in vitro* and *in vivo*.^{250,258,260–266}

Both increased rates of lipid peroxidation and protein carbonylation was reported both in the mother and fetus in experimentally induced diabetic pregnancy.²⁶⁷ Also, increasing evidence has indicated a role for ROS in the development of diabetes-induced malformations in the rodent models.^{260,268}

Further evidence that ROS is involved in diabetes-induced dysmorphogenesis is provided by studies where antioxidant treatment with butylated hydroxytoluene, vitamin E or C resulted in a decline in the occurrence of gross malformations from approximately 25% to less than 8%.²⁶⁹ Increased concentrations of lipid peroxides as a result of ROS damage and reduced concentration of antioxidant vitamin E provides a rationale for developing antiteratogenic treatments for pregnant women with diabetes mellitus. Furthermore, diabetes per se is a state of oxidative stress. Low-density lipoprotein from pregnant diabetic women is more susceptible to oxidation. Embryonic mitochondria have been implicated in mediating ROS-induced teratogenic effect in a diabetic environment, as a consequence of increased production of ROS, decreased capacity to

scavenge ROS or both.^{257,260,261,270,271} Administering antioxidants to embryos in a diabetic environment, both *in vivo* and *in vitro*, significantly reduces the developmental damage.^{257,258,260–265}

Reactive oxygen species and hyperglycemia

Embryos cultured under hyperglycemic conditions show increased formation of ROS and depletion of GSH contents, as well as reduced synthesis of GSH.²⁵⁷ The addition of free radical scavenging enzymes (e.g. SOD, catalase, GPX and N-acetylcysteine, butylated hydroxytoluene) to culture media reduced the incidence of embryonic malformation after hyperglycemia.^{265,268} Hyperglycemia-induced embryonic malformations may be due to increased ROS formation and depletion of intracellular GSH in embryonic tissues.²⁷² GSH depletion and impaired responsiveness of GSH-synthesizing enzyme to oxidative stress during organogenesis may have important roles in the development of embryonic malformations in diabetes.

Pregnancy complicated by poor control of diabetes is associated with a high risk of embryopathies, spontaneous abortions and perinatal mortality attributed to excessive oxidative stress.²⁷³ Both lipid peroxidation and scavenging enzyme activities may be valuable, sensitive indices of fetal/neonatal threat in diabetic pregnancy in humans. Adequate measurements at the time of birth would significantly contribute to clarifying the fetal/neonatal status in a medical and legal context, and may be even of value in altering therapy in newborn infants.

Dysmorphogenesis caused by maternal diabetes is correlated with ROS-induced inhibition of glyceraldehydes-3-phosphate dehydrogenase (GAPDH) in embryos indicating that inhibition of GAPDH plays a causal role in diabetic embryopathy.²⁷⁴

The offspring of women with diabetes mellitus are at increased risk for congenital defects.^{275–281} The incidence and severity of the defects are related to glycemia within the first weeks of pregnancy, during which organogenesis is initiated.^{282–285}

Maternal diabetes also inhibits the expression of PAX-3, an embryonic gene required for neural tube closure caused by increased levels of oxidative stress. Alpha-tocopherol blocks these defects.²⁸⁶

Role of antioxidants

Reactive oxygen species are increased in embryos exposed to excess glucose.^{257,270,287} This could be due both to increased oxidative metabolism and superoxide

generation, or a result of the relative immaturity of the free radical scavenging pathways.^{257,270}

Concentration of antioxidant vitamins is decreased both in the diabetic experimental animals and humans.^{288,289} Studies have indicated a protective effect of orally administered antioxidants, such as vitamin E and butylated hydroxytoluene in pregnant diabetic rats,^{258,261} further implicating the generation of ROS or decreased antioxidant status in the teratogenic process of diabetic pregnancy. Hyperglycemia-induced oxidative stress plays a causal role in diabetes-induced congenital defects. This is confirmed by studies utilizing antioxidants such as vitamin E, vitamin C, butylated hydroxytoluene, GSH precursor, N-acetylcysteine or transgenic over expression of copper/zinc superoxide dismutase.^{250,257,258,262–264,271,287} *In vitro*, embryonic dysmorphogenesis has been shown to be significantly diminished, either by restricting the influx of oxidative substrates, such as pyruvates, to the embryonic mitochondria or by improving the embryonic capacity to scavenge-free oxygen radicals.^{260,268}

Vitamin E supplementation to the diabetic mother can correct both decreased vitamin E levels and substantial mitochondrial high-amplitude swelling in the embryos.^{258,290} Antioxidants have been shown to protect the offspring of diabetic rats against malformations.^{258,261,262,264,291,292} Elevated levels of copper zinc SOD, catalase, GPX and SOD elicit a protective effect against diabetes-associated embryopathy.^{268,271}

CONCLUDING REMARKS

STUDIES ON THE implications of oxidative stress in gynecologic disorders have not received adequate attention, and literature on the molecular mechanism(s) underlying these diseases is sporadic. Oxidative stress in infertility involves poor embryo quality, DNA fragmentation, and poor pregnancy outcome in assisted reproductive programs. Maintaining adequate pro-oxidant-antioxidant equilibrium is also important in other gynecologic diseases, such as endometriosis, pre-eclampsia, maternal diabetes, embryopathies and pregnancy. The rise in antioxidants is probably of a compensatory nature responding to the increased peroxide load and reflects severity of the disease. Multi-center clinical trials with large number of patients are needed, and supplementation with vitamins in all pregnant women with pre-eclampsia should be considered. Clinical studies, aiming at characterization of the maternal and fetal oxidative stress in relation to the fetal outcome, should also be conducted.

From our review of the existing literature, we conclude that the importance of oxidative stress in reproduction is just beginning to be appreciated and studied.

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