

# Role of reactive oxygen species in female reproduction and the effects of antioxidant supplementation – Part 2

ASHOK AGARWAL, SAJAL GUPTA

Center for Advanced Research in Human Reproduction, Infertility, and Sexual Function, Glickman Urological Institute and Department of Obstetrics-Gynecology; The Cleveland Clinic Foundation, 9500 Euclid Avenue, Desk A19.1, Cleveland, OH 44195, USA

## INTRODUCTION

Oxidative stress (OS) influences female reproduction. This article reviews the importance of OS at parturition, in preterm labor, gestational diabetes and fetal congenital defects and discusses its effects on neonatal outcomes. OS plays a role in age-related fertility decline and hormonal changes at menopause. The article addresses the epidemiologic studies on dietary antioxidant intake and its relationship to risk of ovarian cancer. Strategies devised to overcome oxidative stress in natural and assisted conception are also discussed. This article also highlights the combination intervention strategy of vitamin C and vitamin E supplementation in the prevention of pre-eclampsia.

## OXIDATIVE STRESS AT PARTURITION

Term labor triggers a compensatory elevation of the nonenzymatic antioxidant reserves in the fetal red blood cell compartment<sup>1</sup>. This protects the neonate from the hyperoxia encountered at birth. Antioxidant reserves in preterm neonates are reduced, rendering them susceptible to free radical-induced damage such as retinopathy and bronchopulmonary dysplasia<sup>2,3</sup>. Lipid peroxidation and protein oxidation products, which are markers of oxidative stress, have been studied in cord blood to assess the influence of the mode of delivery (i.e., vaginal delivery or caesarean section) and gestational age in inducing OS in the fetus<sup>4</sup>. Uncomplicated term labor, term intrauterine growth retardation and pre-eclampsia were associated with elevated cord blood malondialdehyde (MDA) levels, a marker of oxidative stress<sup>5-7</sup>. The presence of elevated levels of MDA in neonates born via vaginal delivery and in those born to mothers with pre-eclampsia is reflective of OS. A randomized controlled trial found that cord blood protein carbonyl levels, a biochemical marker of OS, were significantly lower in preterm infants than in term infants<sup>4</sup>. The lower protein carbonyls in very low birth weight infants (VLBW, <1500 g) may be due to localized OS (e.g., in the lung or retina).

## ROLE OF OXIDATIVE STRESS IN PRETERM LABOR

Prematurity is an important cause of neonatal deaths and long-term morbidity such as developmental delays, blindness and cerebral palsy<sup>8</sup>. The production of reactive oxygen species (ROS), prostaglandins, proinflammatory cytokines and proteases has been implicated in the initiation of term and preterm labor<sup>9</sup>. Microbial invasion is seen in 10 percent of patients with preterm labor and in 38 percent of patients with preterm premature rupture of membranes. OS plays a role in the etiology of chorioamnionitis (CAM). In CAM, there is increased NADP (H)

oxidase- activity-an ROS generating enzyme. CAM is a leading cause of preterm labor and causes the up regulation of COX-2 (cyclooxygenase-2) enzyme in the placenta and prostaglandin synthesis<sup>10</sup>. One study reported that 4 hydroxy-2-nonenal (a marker of OS) was associated with increased expression of COX-2 and prostaglandin E2 in the placenta.

Metalloproteinases are a group of endopeptidase enzymes that cleave the extracellular matrix and are activated in several inflammatory processes. The balance between prooxidants and antioxidants determines the matrix metalloproteinase activity of the amniotic membranes<sup>11</sup>. Metalloproteinase activity was found to be increased directly by superoxide anion, a byproduct of macrophages and neutrophils<sup>11</sup>.

## OXIDATIVE STRESS AND NEONATAL OUTCOMES

OS has been thought to play a role in many illnesses affecting preterm infants, including bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP) and hypoxic ischemic encephalopathy<sup>12,13</sup>. Both enzymatic and nonenzymatic scavenging antioxidants are deficient in preterm infants<sup>3</sup>. The best approach to preventing the free radical-induced injuries in preterm infants is by providing optimum oxygen therapy and avoiding hyperoxia. Significantly elevated concentrations of 8-iso-prostaglandins F2 $\alpha$ , a product of lipid peroxidation, were detected in cord blood from singleton pregnancies complicated by moderate or thick meconium-stained liquor<sup>14</sup>. Management of OS with amnioinfusion may be beneficial in patients with meconium stained liquor because it lowers lipid peroxidation levels, a marker of oxidative stress. OS adversely affects fetal outcomes in preterm infants, and in term infants, it is associated with fetal distress. There is increased oxidative stress associated with gestational diabetes. OS may have a role in the embryo and fetal development in-utero. Studies have investigated utilizing scavenging antioxidants in neutralizing the OS. Studies in mice have shown that the antioxidant  $\alpha$ -tocopherol decreased the occurrence of valproic acid and hyperglycemia induced neural tube defects.

## OXIDATIVE STRESS AND DIABETIC PREGNANCY

Increased lipid peroxidation and reduced levels of antioxidants may be partially responsible for diseases that complicate pregnancies. Elevated concentrations of thiobarbituric acid reactive substances (TBARS) and reduced antioxidant enzymes (i.e. copper zinc superoxide dismutase (Cu ZnSOD), catalase and glutathione peroxidase) were demonstrated in hemolysed erythrocytes from patients with pregnancies that were complicated with well-controlled diabetes<sup>15</sup>. The parameters of

oxidative stress, TBARS and antioxidant capacity were measured in all three trimesters of pregnancy<sup>15,16</sup>. A large study on 70 patients with pregestational diabetes demonstrated an association between poor fetal outcome and elevated peroxide levels and reduced antioxidant capacity<sup>17</sup>.

## ROLE OF OXIDATIVE STRESS IN MENOPAUSE AND AGE RELATED FERTILITY DECLINE

ROS may play a role in age-related decreases in estrogen production. Expression of both superoxide dismutase and glutathione peroxidase are decreased in the ovary from the premenopausal to menopausal period<sup>18</sup>. Levels of superoxide dismutase and glutathione peroxidase are significantly and positively correlated with aromatase enzyme activity. Reduced aromatase levels resulted in diminished estrogen production, which is associated with menopause.

Free radical-induced damage may be responsible, at least partially, for the age-related decline in follicle reserves in terms of number and quality<sup>19</sup>. Specifically, early mitochondrial damage, as seen in the ultra structural changes in the ovary, may be characteristic of free radical-induced damage.

Plasma concentrations of 8-epi-prostaglandin F<sub>2</sub> $\alpha$ , another biomarker of oxidative stress, declined in postmenopausal women after a short course of estrogen-progesterone hormone therapy<sup>20</sup>.

## DIETARY ANTIOXIDANTS AND FEMALE REPRODUCTION

The importance of antioxidant nutrient balance in pregnant women is being realized in the literature. Intrauterine fetal growth may be influenced by the status of OS in the mother. A recent report indicates that maternal plasma levels of vitamin C and E were significantly associated with the birth weight and length of the term live-born infants<sup>21</sup>. Newborns born to mothers with the high levels of vitamins C and E in plasma had the highest birth weights and heights.

In a large prospective cohort study, a three-fold higher risk of developing gestational diabetes was demonstrated in women with low levels of plasma ascorbic acid<sup>22</sup>. Antioxidant intake during pregnancy influences the incidence of congenital anomalies. Significantly lower dietary intake of vegetable protein, beta-carotene, ascorbic acid and  $\alpha$ -tocopherol was demonstrated in a group of women who gave birth to children with orofacial clefts<sup>23</sup>.

A positive association between maternal antioxidant intake during pregnancy and the risk of developing atopy, wheeze and eczema during early childhood was found in a large cohort study of 2000 women who were recruited early in pregnancy<sup>24</sup>.

In a case-controlled study conducted in China, higher dietary vitamin C, vitamin E and carotene intake lowered the risk of ovarian cancer with a significant dose-response relationship<sup>25</sup>. Another epidemiologic study was conducted consisting of 2135 population controls and 442 incidental cases of ovarian cancer. The data revealed that women who took vitamin E, beta-carotene and B-complex vitamin supplements for 10 years or more had odds ratios (95% CIs) of 0.49 (0.30-0.81), 0.31 (0.11-0.91), and 0.61 (0.36-1.05) for developing ovarian cancer, respectively. Women receiving supplementation had lower risk of ovarian cancer<sup>26</sup>.

## ROLE OF ANTIOXIDANTS IN OVERCOMING OXIDATIVE STRESS IN INFERTILITY

Considerable interest has been generated in the use of antioxidants to overcome the adverse and pathological results of

OS. OS can directly damage oocytes in developing follicles, oocytes and spermatozoa in the peritoneal cavity, and embryos in the fallopian tube<sup>27,28</sup> or cause an imbalance in redox, leading to luteal regression<sup>29</sup> and resulting in lack of luteal support to pregnancy. Strategies are being designed to reduce the generation of ROS or to increase the amounts of antioxidants available. The reviewed literature has investigated the use of nutritional supplements and antioxidants like vitamin C supplementation. However, there is lack of consensus on the type and dosage of antioxidants to be used.

Current evidence supports the use of systemic antioxidants for management of selected cases of male infertility<sup>30</sup>. However, few trials have been performed to examine the role of antioxidants in female infertility. Clinical evidence on the benefits of antioxidant supplementation is equivocal.

In a recent randomized controlled, multi-center study, the effect of vitamin C supplementation (750 mg/day) in patients with a luteal phase defect was studied; the pregnancy rate was significantly higher in the treatment group than in the controls<sup>31</sup>. Similarly, concentrations of plasma ascorbic acid,  $\alpha$ -tocopherol, total thiols and erythrocyte glutathione were found to be significantly lower in women with a history of recurrent miscarriages, associated with etiologies like autoimmune etiology, unexplained etiology, and luteal phase defects compared to healthy women<sup>32</sup>.

In a double blind, placebo-controlled pilot study, the impact of a nutritional supplement containing vitamin E, iron, zinc, selenium and L-arginine was examined<sup>33</sup>. Mean mid-luteal progesterone levels increased from 8.2ngm/mL to 12.8ngm/mL, and ovulation and pregnancy rates increased significantly (33% pregnant, vs 0%,  $p < 0.01$ )<sup>33</sup>.

A significant negative association has been reported between duration of smoking and fertilization rates in *in-vitro* fertilization (IVF) procedures<sup>34,35</sup>. Smoking was reported to impair folliculogenesis by inducing oxidative stress in the preovulatory follicle<sup>36</sup>. Eliminating the smoking factor would help improve fertility and ART outcomes<sup>37</sup>. Because a history of smoking is associated with high concentrations of oxidative stress, the use of *in-vivo* antioxidants can be recommended in infertile women who smoke<sup>36</sup>.

## STRATEGIES TO OVERCOME OXIDATIVE STRESS IN ASSISTED REPRODUCTION

Oxidative stress is involved in defective embryo development<sup>27,38</sup>. In human IVF/intracytoplasmic sperm injection (ICSI) procedures, the clinical pregnancy rates have remained unchanged at 30-40 percent<sup>39</sup>. It is hypothesized that the altered redox state in *in vitro* conditions may play a role in poor assisted reproduction treatment (ART) outcomes, and controlling oxidative stress may improve ART outcomes<sup>27,40</sup>.

Fertilization and embryo development *in vivo* occur in an environment of low oxygen tension<sup>41</sup>. During ART procedures, it is important to avoid conditions that promote ROS generation and expose gametes and embryos to ROS. During culture, low oxygen tension improves the implantation and pregnancy rate better than high oxygen tension<sup>42</sup>. Similarly, higher implantation and clinical pregnancy rates are reported when antioxidant supplemented media is used rather than standard media without antioxidants. Metal ions can sometimes result in the production of oxidants. Metal ions can also increase the production of ROS directly through the Haber-Weiss reaction. It may be useful to add metal ion chelating agents to culture media to decrease the production of oxidants<sup>42</sup>.

Adding ascorbate during cryopreservation reduces the levels of hydrogen peroxide and thus the oxidative distress in mammalian embryos<sup>43</sup>. As a consequence, embryo development improved with enhanced blastocyst development rates.

Mechanical removal of ROS in IVF/ET has been studied<sup>44</sup>.

The rinsing of cumulus oophorus can overcome the deleterious effects of ROS in patients with ovarian endometriosis<sup>44</sup>. ROS has deleterious effects on both the oocyte and embryo quality in patients with endometriosis. Levels of TNF- $\alpha$  cytokines and ROS are increased in the peritoneal fluid of patients with endometriosis and unexplained infertility, and rinsing of cumulus cells removes the cytokines and free radicals.

Spermatozoa are particularly susceptible to ROS-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids and their cytoplasm contains low concentrations of the scavenging enzymes<sup>45</sup>. Sperm preparation methods affect ART outcomes. Sperm preparation by centrifugation may be associated with generation of ROS. It has been reported that seminal plasma is rich in antioxidants and protects the spermatozoa from DNA damage and lipid peroxidation<sup>46</sup>. Supplementation of IVF media with N-tert-butyl hydroxylamine and SOD/catalase mimetics was reported to block the breakdown of sperm chromatin<sup>47</sup>.

Reports suggest that a prolonged sperm-oocyte co-incubation time (16-20 hours) increases the generation of ROS. Two prospective randomized controlled studies have advocated using a shorter sperm-oocyte co-incubation time<sup>48,49</sup>. Co-incubation times of 1-2 hours resulted in better quality embryos and significantly improved fertilization and implantation rates<sup>50</sup>. The composition of the media utilized for IVF can also influence on the oxidant status of the oocytes and pre-implantation embryos. Current evidence shows that supplementation of the media with antioxidants, disulphide reducing agents or divalent chelators of cations may be beneficial to embryos under *in vitro* conditions<sup>27</sup>.

## INTERVENTIONS TO OVERCOME OXIDATIVE STRESS IN PRE-ECLAMPSIA

Currently, there are no accepted interventions for prevention of pre-eclampsia<sup>51</sup>. Randomized controlled trials have investigated the benefits of antioxidant supplementation in patients with pre-eclampsia. No beneficial results were observed in patients with established pre-eclampsia. The results of the two trials indicated that supplementation should be started early in pregnancy, around 16-20 weeks<sup>52,53</sup>. Antioxidant supplementation with vitamin C and vitamin E-begun at 16-22 weeks of pregnancy reduced the incidence of pre-eclampsia in the supplement group by more than 50 percent<sup>54</sup>. Recently, the DAPIT (diabetes and pre-eclampsia intervention trial) has been instituted as a multi-centered, randomized double-blind placebo controlled trial. In this trial, antioxidant supplementation starts at 8-22 weeks of pregnancy in patients with type-1 diabetes. This study will examine beneficial effects of supplementation in preventing pre-eclampsia will be investigated in this study<sup>55</sup>.

## CONCLUSION

A review of the existing literature elucidates the role of OS in modulating a gamut of physiological functions and its role in pathological processes affecting the female reproductive tract. Strategies to help overcome OS in natural and assisted conception are needed. It is important to further elucidate the role of OS in unexplained infertility and recurrent early pregnancy losses and thus design strategies to overcome its adverse effects. Large randomized double-blind case-controlled studies are needed to assess the safety and efficacy of antioxidant supplements such as vitamins C and E in pre-eclampsia, miscarriage, preterm labor and diabetic pregnancy and to validate results of published studies (case control, observational).

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