

# Role of reactive oxygen species in female reproduction.

## Part I. Oxidative stress: a general overview

ASHOK AGARWAL\*  
SAJAL GUPTA

\* Corresponding author

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

### OXIDATIVE STRESS: GENERAL OVERVIEW

A complex interaction, of prooxidants and antioxidants, modulates the generation of oxidative stress. Free radicals are defined as any species with one or more unpaired electrons in the outer orbit<sup>1</sup>. The generation of the highly reactive free radicals is an inherent feature of normal cellular metabolism<sup>2</sup>. As more research is done focusing on the reactive oxygen species, there is new light being shed on the role of these radicals in physiological functions and whenever there is an excess of the free radicals, they precipitate pathologies in the female reproductive tract.

There are two types of free radicals: reactive oxygen species and reactive nitrogen species. The main radicals in the reactive oxygen species are the superoxide radical, hydrogen peroxide, hydroxyl and the singlet oxygen radicals. There is an array of protective mechanisms that neutralize the oxidants or free radicals. The non-enzymatic antioxidants are vitamin C, taurine, hypotaurine, cysteamine and glutathione and these protect against extraneous ROS. The enzymatic antioxidants are SOD, catalase, glutathione peroxidase and glutaredoxin.

Oxygen radicals and reactive oxygen species play a dual role to in the female reproductive tract, in that they can be both physiologic as well as pathologic. The pathologic effects are exerted by various mechanisms including lipid damage, inhibition of protein synthesis, DNA damage, mitochondrial alterations and depletion of ATP<sup>2-7</sup>. There are some studies reflecting some understanding of how the free radicals affect a gamut of physiologic functions in female reproduction. These are oocyte maturation, ovarian steroidogenesis, ovulation, luteolysis, luteal maintenance in pregnancy, implantation, compaction and blastocyst development<sup>8-12</sup>.

### ROLE OF OXIDATIVE STRESS IN FEMALE REPRODUCTIVE TRACT

Various biomarkers of oxidative stress have been studied in the female reproductive tract. Reactive oxygen species and the transcripts of the various antioxidant enzymes have been localized and different studies have confirmed their presence in the female reproductive tract. (Refer Table-1). Reactive oxygen

### ABSTRACT

*Oxidative stress occurs when there is an elevated concentration of intracellular reactive oxygen species in a steady state condition. When the balance between reactive oxygen species and antioxidants is tipped towards overabundance of ROS, oxidative stress results. Oxidative stress has been implicated in the etiology of multiple diseases like atherosclerosis, Alzheimer's and ageing. This article addresses and reviews the literature on the role of free radicals in female reproduction. Free radicals influence the entire reproductive span of a woman's life and even during menopause. Review of the literature emphasizes that oxidative stress acts as a mediator in the modulation of important ovarian functions, endometrial cyclical changes, embryo development, tubal functions, pregnancy, and its complications such as abortions, recurrent pregnancy losses, preeclampsia, and gestational diabetes. Oxidative stress influences the outcome of natural and assisted fertility and plays a role in the etiopathogenesis of various causes of infertility i.e. endometriosis. Infertility is a problem with huge magnitude, and multiple etiological factors. The etiology of unexplained infertility and recurrent pregnancy loss remains unclear and is a scientific challenge. The treatment is mainly empirical. Oxidative stress may be a piece in this puzzle. Pregnancy is a state of oxidative stress as revealed by number of studies demonstrating elevated levels of the oxidative stress biomarkers. The redox state, influences the maintenance of uterine quiescence in pregnancy, via the paracrine effects of nitric oxide radical on the myometrium, inhibiting uterine contractions. Oxidative stress at parturition up regulates the antioxidant reserves of the neonate, but its role in initiation of labor is not known. Preterm fetuses are susceptible to free radical induced injuries, retinopathy, and bronchopulmonary dysplasia. Currently available studies on antioxidant supplementation have been reviewed. In this paper we will discuss the role of various antioxidants both in-vivo and in-vitro, in overcoming oxidative stress affecting female reproductive tract pathologies. Clinical evidence on the role of antioxidants in modulating disease outcomes in female reproduction is equivocal. Trials investigating combination intervention strategy of vitamin E and vitamin C supplementation in preventing preeclampsia are highlighted. There is ongoing research investigating the exact mechanisms by which oxidative stress causes pathological processes affecting female reproduction i.e. infertility, abortions, preeclampsia and fetal embryopathies and to design strategies for surmounting oxidative stress.*

**Keywords:** Oxidative stress, reactive oxygen species, antioxidants, female infertility, female reproduction, abortion, preeclampsia and fetal embryopathy

species may act as important mediators in hormone signaling, ovarian steroidogenesis, germ cell function, corpus luteum formation and luteolysis.

## OXIDATIVE STRESS AND OVARIAN FOLLICULOGENESIS

Reactive oxygen species most likely play a regulatory role in oocyte maturation, folliculogenesis, ovarian steroidogenesis, and luteolysis. Immunostaining to study SOD<sup>10,13</sup> expression and ELISA to assess the levels of SOD in follicular fluid have been reported<sup>14</sup>. There is intense staining of the theca interna cells once the antral cavity begins to form. Theca interna cells may act as important protectors of the oocyte from oxidative stress during oocyte maturation. CuZnSOD was localized in the developing follicles, granulosa cells of the graafian follicles and in postovulatory follicles<sup>15</sup>. Markers such as superoxide dismutase, Cu-Zn superoxide dismutase and Mn superoxide dismutase, glutathione peroxidase,  $\gamma$  glutamyl synthetase and lipid peroxides have been investigated<sup>9-10,16</sup>. These markers have been determined by immunohistochemical localization, m-RNA expression and thiobarbituric acid method respectively. The expression of various biomarkers of oxidative stress have been demonstrated in normal cycling human ovaries<sup>9,14</sup>. All follicular stages were examined for the expression of the SOD including primordial, primary, preantral, nondominant antral follicles in follicular phase, dominant follicles and atretic follicles<sup>9</sup>. There is a delicate balance between reactive oxygen species and the antioxidant enzymes in the ovarian tissues. The antioxidant enzymes neutralize ROS production and protect oocyte and the embryo from oxidative stress.

## ROLE OF OXIDATIVE STRESS IN CYCLICAL ENDOMETRIAL CHANGES

Oxidative stress is involved in the modulation of cyclical changes in the endometrium. Changes in the expression of superoxide dismutase in the endometrium have been studied. Superoxide dismutase is an enzyme involved in scavenging the superoxide radical and protecting the cells from oxygen radical toxicity. The levels of SOD increase and the ROS levels increase in the endometrium in the late secretory phase just before menstruation indicating that these changes in the level of expression indicate involvement in menstruation<sup>13</sup>. Estrogen and progesterone withdrawal led to increased expression of

cyclooxygenase -2 mRNA and increased prostaglandin F2 $\alpha$  synthesis in endometrial cells cultured in vitro. These effects were proposed to be ROS mediated nuclear factor kappa B (NF $\kappa$ B) activation<sup>17</sup>.

## OXIDATIVE STRESS AND EARLY EMBRYO DEVELOPMENT

Oxidative stress is involved in the etiopathogenesis of defective embryo development<sup>11</sup>. Oxidative state, or minimal levels of oxidative stress may be beneficial for embryo growth and development. An increase in ROS production was found to lead to arrest of embryo development at two-cell stage<sup>18</sup>.

Reactive oxygen species may originate in the embryo or from the extraneous factors<sup>19</sup>. There are many factors in the in vitro culture conditions, which may result in oxidative stress having detrimental effects on the embryo. ROS in the culture media may originate from the oocytes, cumulus cell mass and spermatozoa used for insemination<sup>20</sup>. It has been reported that redox modulates the expression of key transcription factors and can alter gene expression during embryo development<sup>21</sup>. In the IVF setting strategies to reduce ROS production like addition of free radical scavengers and lowering the oxygen tension are important for improving the fertility potential in assisted reproduction.

## ROLE OF ROS IN FEMALE INFERTILITY

Reactive oxygen species are a double-edged sword, which have been localized in the female reproductive tract in various animal and human studies. The review has already discussed the role ROS plays in modulating the cyclical ovarian and endometrial changes. There is some understanding of the role of oxidative stress in infertility and its causative factors like, endometriosis, unexplained infertility and tubal factor infertility<sup>22-25</sup>. Peritoneal macrophages are activated in patients with endometriosis, which is associated with increased scavenger receptor activity<sup>26-27</sup>. The activated macrophages are a source of increased ROS generation in patients with endometriosis<sup>28</sup>. In contradiction, two other studies have reported no significant difference in ROS concentrations between patients with endometriosis and healthy controls<sup>29,30</sup>. These studies suggested that oxidative stress might be a localized phenomenon that occurred at the site of bleeding. In one study elevated ROS were demonstrated in women undergoing evaluation for unexplained infertility compared with

Table -I Biomarkers oxidative stress in female reproduction

Reproductive tract site	Biomarkers	References
Ovary	Superoxide dismutase, CuZn SOD, Mn SOD, Glutathione peroxidase, conjugated dienes, lipid peroxides, Thiobarbituric acid reactive substances, Glutaredoxin, Oxidative DNA adducts, Follicular fluid NO levels and TAC	8, 10, 15, 50-61
Endometrium	Superoxide dismutase, iNOS and eNOS.	62-70
Fallopian Tubes	ROS, Superoxide dismutase, iNOS and eNOS	25, 52, 71
Embryo	Reactive oxygen species, total antioxidant capacity	11, 20, 67, 72-75
Placenta	Lipid hydroperoxides, Intracellular ROS, Total antioxidant capacity and Oxidative DNA adducts-8-hydroxy 2-deoxyguanosine.	76-79
Peritoneal fluid (in Endometriosis)	Oxidized lipoproteins, Intraperitoneal fluid ROS, and Total antioxidant capacity	80-81

fertile women undergoing laparoscopy for tubal ligation<sup>29</sup>. Polak *et al.* found that the concentrations of antioxidants in the peritoneal fluid were lower and the lipid peroxidation products were elevated in patients with idiopathic infertility. This reflects a perturbed redox state in patients with unexplained infertility<sup>31</sup>.

## OXIDATIVE STRESS AND ASSISTED REPRODUCTION

An imbalance in the redox state of the developing embryo, as a result of suboptimal culture conditions leads to altered gene expression and impaired Adenosine triphosphate generation<sup>3</sup> and which can impair placental and embryo growth<sup>12</sup>. Strategies to overcome oxidative stress are aimed at minimizing the exposure of gametes to environments that generate free radicals. Spermatozoa are the exogenous source of ROS. Reducing the incubation time of oocytes and spermatozoa has been reported as beneficial in overcoming oxidative stress by some authors<sup>32-33</sup> and others have reported no benefits<sup>34</sup>.

## ANTIOXIDANT AND PRO-OXIDANT BALANCE IN ABORTION

Abnormal placentation has been implicated in the pathogenesis of preeclampsia and miscarriage<sup>35</sup>. Preeclampsia is unique to human species and miscarriage is very rare in other species<sup>36</sup>. Abnormal placentation leads to placental oxidative stress with resultant detrimental effects on the trophoblast and has been proposed as a mechanism causing abortion<sup>37</sup>. A sharp peak in the expression of the markers of oxidative stress in the trophoblast was detected in normal pregnancies and this oxidative burst if excessive was speculated as a cause of early pregnancy loss<sup>38</sup>.

The etiology of recurrent pregnancy loss remains unclear and is a scientific challenge. Oxidative stress may have a role in the etiology of recurrent pregnancy loss with no known etiology. Glutathione and glutathione transferase family of enzymes have been investigated in patients with recurrent abortions<sup>39-40</sup>.

Glutathione and glutathione peroxidase are both antioxidants that neutralize the free radicals and lipid peroxides to maintain the intracellular homeostasis and redox balance.

The etiology of recurrent pregnancy losses is multifactorial and involves genetic and environmental factors<sup>41</sup>. In a large case controlled study, gene polymorphisms of enzymes from the glutathione family<sup>40</sup> were studied in a group of women with recurrent pregnancy loss. The authors reported that elevated risk of recurrent pregnancy loss was associated with the GSTM1 (mu glutathione s-transferase family) genotype null polymorphism. Polymorphism of the bio transformation enzymes like glutathione transferase have also been investigated in patients with preeclampsia<sup>43</sup> and patients with endometriosis<sup>42</sup>.

Elevated glutathione levels in pregnant patients with a history of recurrent pregnancy loss were associated with poor outcomes i.e. abortion<sup>39</sup>.

## OXIDATIVE STRESS AND PREGNANCY

Elevated levels of lipid peroxides and vitamin E have been reported in pregnancy<sup>44-45</sup>. Reduction in antioxidant levels and elevated peroxidation product levels were demonstrated in pregnancy<sup>46,48</sup>. Pregnancy is associated with an inflammatory response characterized by leukocyte activation<sup>49-50</sup>. There is an increase in the intracellular reactive oxygen species in leucocytes seen in the third trimester of pregnancy<sup>50</sup>. The leukocyte activation response was exacerbated in pregnancies complicated by preeclampsia<sup>49</sup>.

## CONCLUSION

Review of the existing literature demonstrates the role of oxidative stress in modulating a gamut of physiological functions and its role in pathological processes affecting the female reproduction. Oxidative stress influences a host of reproductive processes in a woman's life. The role of oxidative stress is becoming increasingly important as there is new cumulative evidence which suggests that oxidative stress is involved in conditions such as abortions, preeclampsia, hydatidiform mole, fetal embryopathies, preterm labor and intrauterine growth retardation, all of which lead to an immense burden of maternal and fetal, morbidity and mortality. It is also important to further elucidate the role of oxidative stress in unexplained infertility and recurrent early pregnancy losses and thus design strategies to overcome its adverse effects. Preeclampsia is a disease with a huge magnitude of disease burden, affecting 10% of first time pregnancies. Oxidative stress has been found to play a pivotal role in its pathogenesis. There is ongoing debate on the role of antioxidants in modifying the disease outcomes. The results of the studies reviewed need to be validated by larger randomized, multicenter, double blind and case-controlled trials.

## Abréviations

Cu Zn SOD( Copper Zinc superoxide dismutase), eNOS ( endothelial nitric oxide synthase), ELISA(enzyme linked immunsorbant assay), iNOS ( inducible nitric oxide synthase), Mn SOD (Manganese superoxide dismutase), NFκB ( Nuclear factor kappa B), ROS (reactive oxygen species), SOD (superoxide dismutase), TAC( total antioxidant capacity)

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