Female Infertility and Antioxidants

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INTRODUCTION

- Reactive oxygen species (ROS) : modulate cellular functions
- oxidative stress (OS) : impair the intracellular milieu →diseased cells or endangered cell survival
- ROS →excessive levels can result in precipitous pathologies affecting female reproduction.
- The oxidant status influence early embryo development by modifying the key transcription factors, hence modifying gene expression.

WHAT IS OXIDATIVE STRESS?

- Oxidative stress arises from an **imbalance** between prooxidant molecules generated from aerobic metabolism and protective antioxidants.

(*Pro-Oxidants*)

- ROS :
  - formed endogenously during aerobic metabolism and as a result of various metabolic pathways of oocytes and embryos or as part of the body's defense mechanisms.
  - arise from exogenous sources, *such as alcohol, tobacco, and various environmental pollutants*.
  - include hydroxyl radicals, superoxide anion, hydrogen peroxide, and nitric oxide (NO)

- Several biomarkers indicative of redox status, including
  - ***Superoxide dismutase*** (SOD), ***glutathione peroxidase*** (GSH-Px), ***lipid peroxides, and nitric oxide***
  - identified within the ovary, endometrium, fallopian tubes, embryo, placenta, and the peritoneal fluid of women.
- controlled levels: free radicals are capable of exerting physiological effects and mediating processes such as:
  - *tissue remodeling, hormone signaling, oocyte maturation, folliculogenesis, tubal function, ovarian steroidogenesis, cyclical endometrial changes, and germ cell function*
- pathological levels: significant damage to cell structures.

(*Antioxidants*)

- Under normal conditions, antioxidants act to oppose ROS production, scavenge existing free radicals, and promote the repair of ROS-induced damage to cell structures
- Nonenzymatic antioxidants:
  - ***vitamin C, vitamin E, selenium, zinc, beta carotene, carotene, taurine, hypotaurine, cysteamine, and glutathione.***
- Enzymatic antioxidants:
  - ***SOD, catalase, GSH-Px, glutaredoxin and glutathione reductase***
- The degree of antioxidant defense present is often expressed as total antioxidant capacity (TAC)

- A disruption balance between antioxidants and pro-oxidant molecules → OS.
- the generation of ROS and other radical species overrides the scavenging capacity by antioxidants *(either due to the excessive production of ROS or an inadequate availability of antioxidants)* → OS arise!!
- oral antioxidant supplementation may serve to prevent and alleviate OS and its contribution to the pathogenesis of obstetrical disease such as:
  - preeclampsia
  - recurrent pregnancy loss
  - gynecological disorders : PCOS and endometriosis.

OS IN THE FEMALE REPRODUCTIVE TRACT – PHYSIOLOGICAL ROLE OF OS
(Follicle)

- The follicular fluid microenvironment contains leukocytes, macrophages, and cytokines →sources of ROS
- ROS within the follicular fluid plays a role in modulating oocyte maturation, folliculogenesis, ovarian steroidogenesis, and luteolysis

- Studies : nitric oxide radical in the follicular growth and programmed follicular cell death that occur during folliculogenesis
- ***Moderate OS levels are required for ovulation.***
- The final stages of oocyte maturation are associated with fluctuations in cytokines, prostaglandins, proteolytic enzymes, nitric oxide, and steroids → increase ROS →influencing ovarian blood flow and eventually facilitating follicle rupture

- ROS is controlled and kept at physiological levels within the ovary by various antioxidant systems *(catalase, vitamin E, glutathione and carotenoids)*
- SOD: a metalcontaining enzymatic antioxidant characterized in the theca interna cells in the antral follicles → may protect the oocyte from excess ROS during its maturation
- Transferrin: an iron-chelating glycoprotein →suppresses ROS generation
- Vitamin C:
  - a protective effect within the follicle
  - vitamin C deficiency → ovarian atrophy, extensive follicular atresia, and premature resumption of meiosis

- reproductive aging : diminish ROS scavenging ability of antioxidants within the follicular fluid microenvironment.
- Carbone *et al.* : decreased levels of follicular fluid catalase and SOD in older women (lower fertilization rates and decreased blastocyst development)

(Endometrium)

- Estrogen and progesterone withdrawal in endometrial cells → associated with a decrease in SOD activity → unopposed activity of ROS
- Elevated lipid peroxide and decreased SOD in the endometrium during the late secretory phase → modulate endometrial breakdown → menstruation.
- NO is produced by endothelial NO synthase (NOS)
  - mediate EM decidualization and menstruation
  - implicated in the changes seen in the endometrium in preparation for implantation

(Infertility)

- semen samples of infertile men: 25-40% → High levels of ROS biomarkers
- physiological role:
  - normal sperm function, mediating the acrosome reaction, hyperactivation, motility, and capacitation of spermatozoa
- excessive levels
  - immotile or morphologically abnormal spermatozoa and leukocytes.
- Spermatozoa lack the necessary cytoplasmic antioxidant enzymes and are vulnerable to OS-induced DNA damage and apoptosis
- Oral antioxidant supplementation has become standard practice for male infertility

- Excess ROS in the follicle : directly damage oocytes.
- The DNA of oocytes and spermatozoa may be damaged, leading to defective fertilization
- fertilization achieved : OS-induced apoptosis → *embryo fragmentation, implantation failure, abortion, impaired placentation, and congenital abnormalities*
- OS : induce luteal regression and insufficient luteal hormonal support for the continuation of a pregnancy
- The association of OS with various gynecologic and obstetric conditions related to infertility suggests a potential role for oral antioxidant supplementation (Fig. **1**).

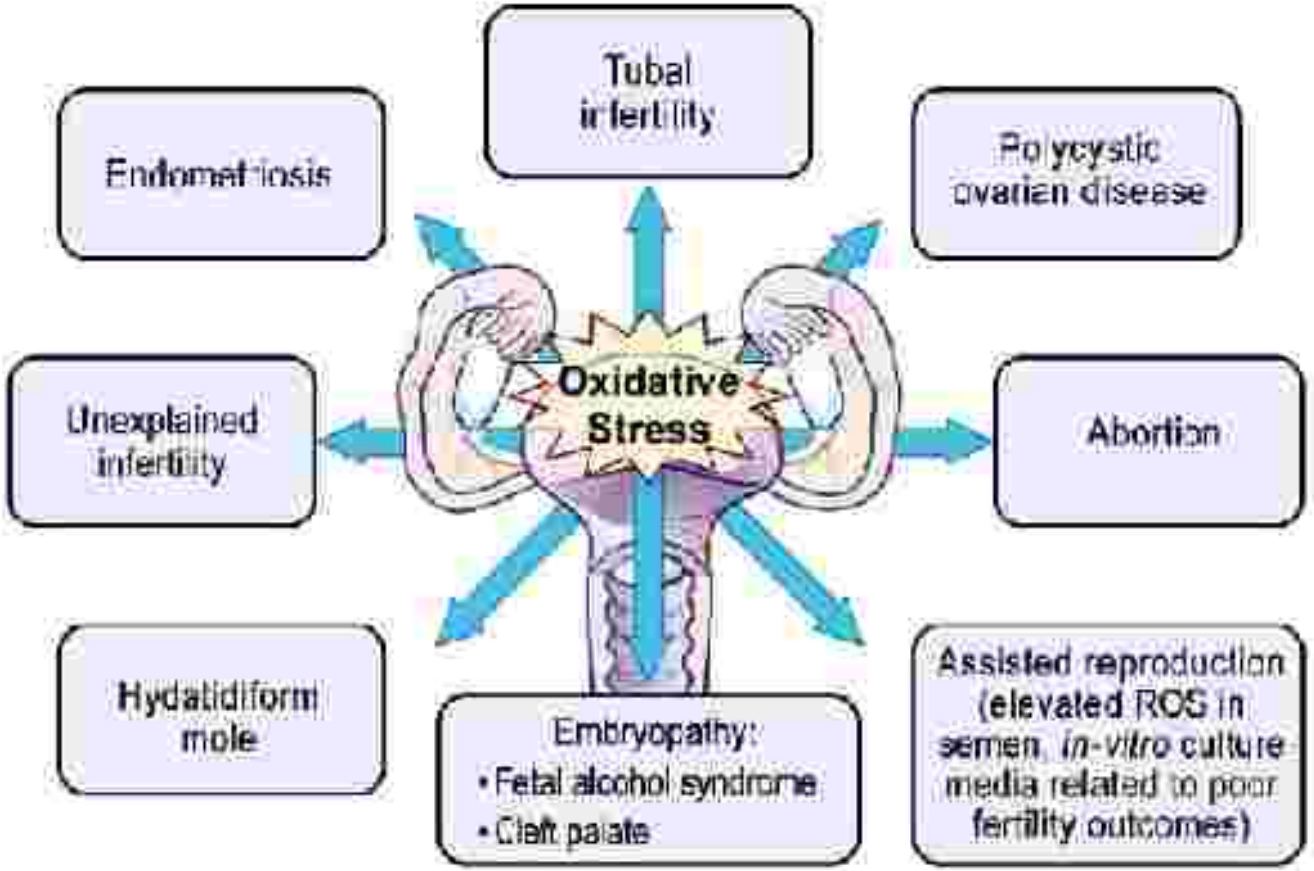

Fig. (1). The role of oxidative stress in obstetric and gynecologic conditions that contribute to infertility.

THE USE OF ANTIOXIDANTS IN TREATMENT OF GYNECOLOGICAL CONDITIONS

(Polycystic Ovarian Syndrome)

- an anovulatory cause of infertility : 6-10% of premenopausal women
- hyperandrogenism, hirsutism, oligomenorrhea or amenorrhea.
- Oxidative stress : implicated in mediating the insulin resistance and increase in androgens seen in these patients

- Kuscu *et al. :*
  - upregulated SOD activity in patients with PCOS
  - Insulin resistance and hyperglycemia are established as factors that increase oxidative stress
- Fulghesu *et al.*
  - N-acetyl-cysteine (NAC) →to replenish stores of the anti-oxidant glutathione, on insulin secretion and peripheral insulin resistance in subjects with PCOS
  - NAC treatment :
    - improve parameters of glucose control in hyperinsulinemic patients.
    - Insulin levels were reduced
    - increased peripheral insulin sensitivity

- Zhang *et al.* used methods of chemicalorimetry to measure and compare levels of serum lipid peroxides (LPO), MDA, SOD, vitamin E, and vitamin C in patients with PCOS and normal women
  - PCOS :
    - serum LPO and MDA were significantly higher
    - Levels of vitamin E, vitamin C, and SOD were lower
  - 3 months of oral ethinylestradiol and cyproterone acetate tablets, an anti-androgenic oral contraceptive often used to treat hirsutism associated with PCOS:
    - MDA and LPO levels decreased
    - vitamin E, vitamin C, and SOD levels increased
  - ***therapy may alleviate the symptoms of PCOS through both its anti-androgenic and anti-oxidant actions.***

(*Endometriosis*)

- Peritoneal fluid containing ROS-generating iron, macrophages, and environmental contaminants → disrupt the prooxidant/antioxidant balance → increased proliferation of tissue and adhesions
- ROS : promote the growth and adhesion of endometrial cells in the peritoneal cavity, contributing to the pelvic anatomical distortion known to cause infertility in endometriosis
- OS : promoting angiogenesis in ectopic endometrial implants by increasing vascular endothelial growth factor (VEGF) production

- Peritoneal fluid of women with endometriosis :
  - increased ROS generation by activated peritoneal macrophages → cytokines and immune mediators such as NO → increasing the OS in the peritoneal fluid
  - insufficient antioxidant defense, with lower total antioxidant capacity (TAC) and significantly reduced SOD levels

- RU486- a potent antiprogestational agent with antioxidant activity → decrease the proliferation of epithelial and stromal cells in endometriosis
- Pentoxifylline: a 3',5'-nucleotide phosphodiesterase inhibitor → potent immunomodulatory properties →significantly reduce the embryotoxic effects of hydrogen peroxide
- Curcumin : a polyphenol derived from turmeric (Curcuma longa) with antioxidant, anti-inflammatory, and antiproliferative properties →anti-endometriotic effect *(mouse model)*

- Matrix metalloproteinase-9 (MMP-9) : correlate with severity of endometriosis → curcumin treatment → reverse MMP-9 activity
- Decreased expression of TNF-α : during regression and healing of endometriotic lesions (mouse model).
- Pretreatment of endometriotic lesions with curcumin : prevent lipid peroxidation and protein oxidation within the experimental tissue
- Melatonin :a major secretory product of the pineal gland with anti-oxidant properties.

- Guney *et al. :* evaluated the effects of antioxidant and anti-inflammatory caffeic acid phenethyl ester (CAPE) on experimental endometriosis (rat model)
  - decrease peritoneal MDA levels and antioxidant enzyme activity.
  - Endometriotic lesions were histologically undergo atrophy and regression
- Mier-Cabrera *et al.*
  - women with endometriosis were found to have a significantly lower intake of vitamins A, C, E, zinc, and copper.

- The green tea-containing compound, epigallocatechin gallate **(EGCG)** : a treatment for endometriosis (powerful antioxidant and anti-angiogenic properties)
- Xu *et al. :* eutopic endometrium transplanted subcutaneously into a mouse model → compare the effects of EGCG VS. vitamin E VS. untreated controls
- EGCG :
  - ***significantly downregulated VEGF-A mRNA***
  - ***significantly smaller endometriotic lesions and smaller and more eccentrically distributed glandular epithelium***
- Control: newly developed blood vessels with proliferating glandular epithelium
- vitamin E: not shown to control or decrease angiogenesis

(Unexplained Infertility)

- Elevated levels of ROS that disturb the redox balance within the body may be the root cause of infertility in women who do not have any other obvious cause
- Wang *et al.*
  - compared ROS levels in peritoneal fluid : laparoscopy for infertility vs. fertile (tubal ligation)
  - ***higher levels of ROS exist in unexplained infertility group***

THE USE OF ANTIOXIDANTS TO PROMOTE HEALTHY PREGNANCY

(Preeclampsia)

- Preeclampsia : 5% of all pregnancies, 11% of first pregnancies → high maternal and fetal morbidity and mortality
- increasing evidence that corroborates the role of OS in its etiopathogenesis → observed in patients with preeclampsia
  - *Reduced antioxidant response*
  - *reduced levels of antioxidant nutrients*
  - *increased lipid peroxidation*

- defective placentation → reduced fetoplacental circulation →decreased NO-mediated vascular relaxation → Placental ischemia and hypoxia → ischemic reperfusion injury to the placenta → release of cytokines and prostaglandins → triggers the endothelial cell dysfunction seen in preeclampsia.

- Hypoxia and reperfusion injury → increased expression of xanthine oxidase and NADPH oxidase and increased generation of SOD.

- Poorly perfuse placental tissue, abnormal lipid metabolism, and resultant lipid peroxidation and redox imbalance are established factors that promote the development of preeclampsia
- Placental oxidative stress has been proposed as a promoter of lipid peroxidation and endothelial cell dysfunction.

- There currently is no accepted method of preventing the development of preeclampsia.
- Some trials : use of supplementation with antioxidants vitamin C and vitamin E for prevention.
  - Early intervention at 16–22 weeks of pregnancy with supplementation of vitamin E and C → significant reduction of preeclampsia in the treatment group
- A recent randomized trial : failed to demonstrate any beneficial effects of vitamin C and E supplementation in preventing preeclampsia

(Recurrent Pregnancy Loss)

- 0.5% to 3% of women of reproductive age.
- associated factors: maternal age, genetic factors, endocrinologic factors, anatomic problems, and environmental toxins
- Etiology: chromosomal abnormalities, uterine anatomic anomalies, immunologic disorders such as antiphospholipid antibody syndrome, clotting disorders, and sperm DNA fragmentation
- 50%-60% of recurrent pregnancy losses are considered idiopathic → Oxidative stress ???

- During pregnancy both extracellular and intracellular ROS production increases sharply (from developing embryo)
- the demand for enzymatic antioxidant defense is increased in embryos and oocytes and their tubal and follicular fluid microenvironments to successfully support a pregnancy and the heightened OS it produces
- The sharp peak in the expression of OS markers in the trophoblast in normal pregnancy may result in damage to protein, lipids, and DNA, which may ultimately lead to cell death if this oxidative burst becomes excessive

- The connection between recurrent pregnancy loss and OS
  - increased levels of antioxidants needed to neutralize and scavenge excessive ROS present in women affected by habitual abortion
- Simsek *et al.*
  - evaluated the outcome of deficient antioxidant defense in women with habitual abortion → elevated lipoperoxides and significantly decreased vitamin A, E, and beta carotene in this population compared with the control group.

- The glutathione peroxidase reductase antioxidant system is an ROS scavenger: preventing lipid peroxidation in cells and maintaining intracellular homeostasis and redox balance

- Studies : glutathione concentration and activity → significantly higher in recurrent miscarriage compared with the women with normal pregnancies or in healthy, non-pregnant woman

- several male factors can contribute to OS
- Gil-Villa *et al. :* evaluating and comparing: recurrent pregnancy loss history VS. control group
  - *increase in sperm DNA damage , embryo loss and augmented time to reach pregnancy*

- the role of antioxidant supplementation in women affected by recurrent pregnancy loss:
  - Poor dietary intake of vitamins has been associated with an increased risk of miscarriage
  - Vitamin C and E are two popular vitamins that may have a potential role in alleviating the effects of OS in women affected with miscarriages.

- Vitamin E:
  - protect against OS-related damage and thereby serve as an antioxidant.
  - In a normal pregnancy, vitamin E level naturally increases
  - in an abnormal pregnancy, vitamin E concentrations are lower
- vitamin C levels increase physiologically during pregnancy
- It is necessary to perform an accurate assessment of the appropriate type and dosage of vitamins that can be tolerated without causing deleterious side-effects to the mother and baby

- Szymanski *et al.*
  - women receiving folic acid supplements had better quality oocytes and a higher degree of mature oocytes compared with those who did not receive folic acid supplementation
  - the results of a study by Gindler *et al.* failed to confirm that the consumption of folic acid decrease a woman's risk for miscarriage

- **Antiphospholipid (aPL) antibody syndrome** : autoimmune cause of recurrent pregnancy loss
- OS has been proposed to have a role in the formation of these antibodies
- Omega-3 supplements have been used in prevention of recurrent miscarriage with antiphospholipid syndrome
- Melatonin : a hormone that acts as a powerful agent against ROS → hypothesized to have properties essential for successful pregnancy and prevention of spontaneous abortion

KEY ISSUES

- Oxidative stress (OS) occurs when the generation of reactive oxygen species (ROS) and other radical species overrides the scavenging capacity of antioxidants
- At controlled levels → physiological female reproductive functions: oocyte maturation, folliculogenesis, ovarian steroidogenesis, luteolysis, ovulation, cyclical endometrial changes, and menstruation.
- At higher levels → pathological processes of the female reproductive tract that contribute to infertility and poor pregnancy outcome, such as PCOS, endometriosis, unexplained infertility, preeclampsia, and recurrent pregnancy loss.

- Antioxidant treatment of PCOS: N-acetyl cysteine may improve glucose control and peripheral insulin sensitivity in hyperinsulinemic patients.
- Endometriosis: The antioxidants catalase, RU486, curcumin, melatonin, and catechins → anti-proliferative and anti-angiogenic effects → halting disease progression.
- studies have failed to detect any risk reduction for preeclampsia with vitamin C and vitamin E supplementation.
- The antioxidants folic acid, melatonin, and omega-3 fatty acids (particularly in women with antiphospholid antibody syndrome) → prevent recurrent pregnancy loss → Further studies to confirm the safety and efficacy of these compounds are needed.

Thanks for your attention!