
توکل بر خُدا....

Title

The role of oxidative stress and antioxidants on female reproduction

Oxidative stress

- Oxidative stress (OS), a state characterized by an imbalance between pro-oxidant molecules including reactive oxygen and nitrogen species, and antioxidant defenses
- A certain amount of ROS is needed for the progression of normal cell functions, provided that upon oxidation, every molecule returns to its reduced state.
- Excessive ROS production, however, may overpower the body’s natural antioxidant defense system, creating an environment unsuitable for normal female physiological reactions.

Oxidative stress

Etiology

1. Increased ROS

- **Endogenously generated during the mitochondrial respiratory chain process**
- **From exogenous exposure such as tobacco smoke, alcohol and environmental chemical exposure**

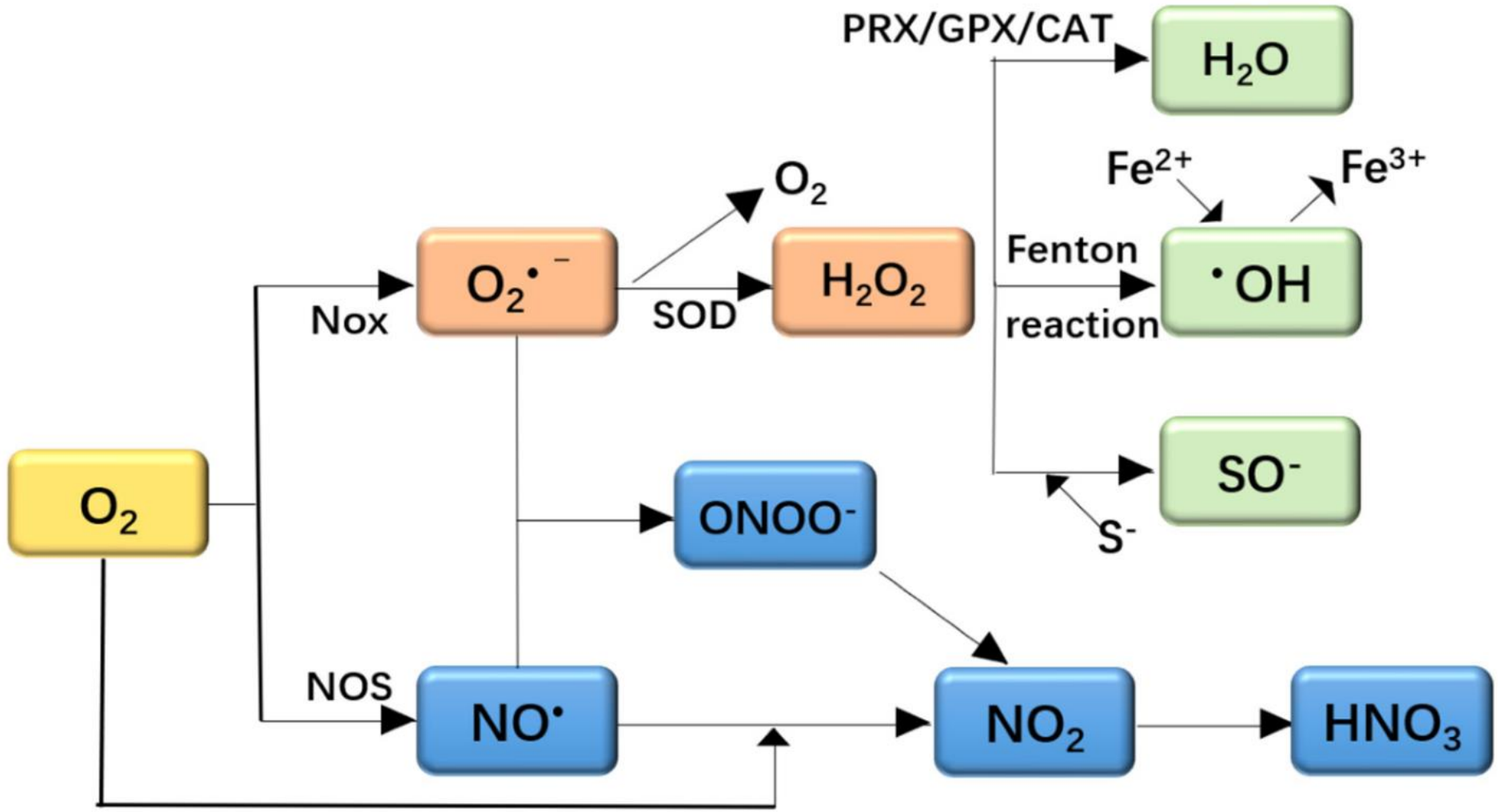
2. Inadequate amount of antioxidants → scavenge free radicals or inhibit excess ROS production

Oxidative stress

- Reactive oxygen species are generated during crucial processes of oxygen (O_2) consumption.
- They consist of free and non-free radical intermediates, with the former being the most reactive. This reactivity arises from one or more unpaired electrons in the atom's outer shell. In addition, biological processes that depend on O_2 and nitrogen have gained greater importance because their end-products are usually found in states of high metabolic requirements, such as pathological processes or external environmental interactions.
- Biological systems contain an abundant amount of O_2. As a diradical, O_2 readily reacts rapidly with other radicals. Free radicals are often generated from O_2 itself, and partially reduced species result from normal metabolic processes in the body. Reactive oxygen species are prominent and potentially toxic intermediates, which are commonly involved in OS.

Oxidative stress

- Reactive nitrogen species include nitric oxide (NO) and nitrogen dioxide (NO_2) in addition to non-reactive species such as peroxynitrite ($ONOO^-$), and nitrosamines.
- In mammals, RNS are mainly derived from NO, which is formed from O_2 and L-arginine, and its reaction with the SO anion, which forms peroxynitrite.
- Peroxynitrite is capable of inducing lipid peroxidation and nitrosation of many tyrosine molecules that normally act as mediators of enzyme function and signal transduction.


PRX/GPX/CAT
H_2O
Fe^{2+}
Fe^{3+}
O_2
Fenton
reaction
$\cdot OH$
Nox
$O_2^{\bullet -}$
SOD
H_2O_2
SO^-
S^-
O_2
$ONOO^-$
NOS
$NO^{\bullet}$
NO_2
HNO_3

Oxidative stress

- It has been identified to play a key role in the pathogenesis of subfertility in both males and females.
- The adverse effects of OS on sperm quality and functions have been well documented.

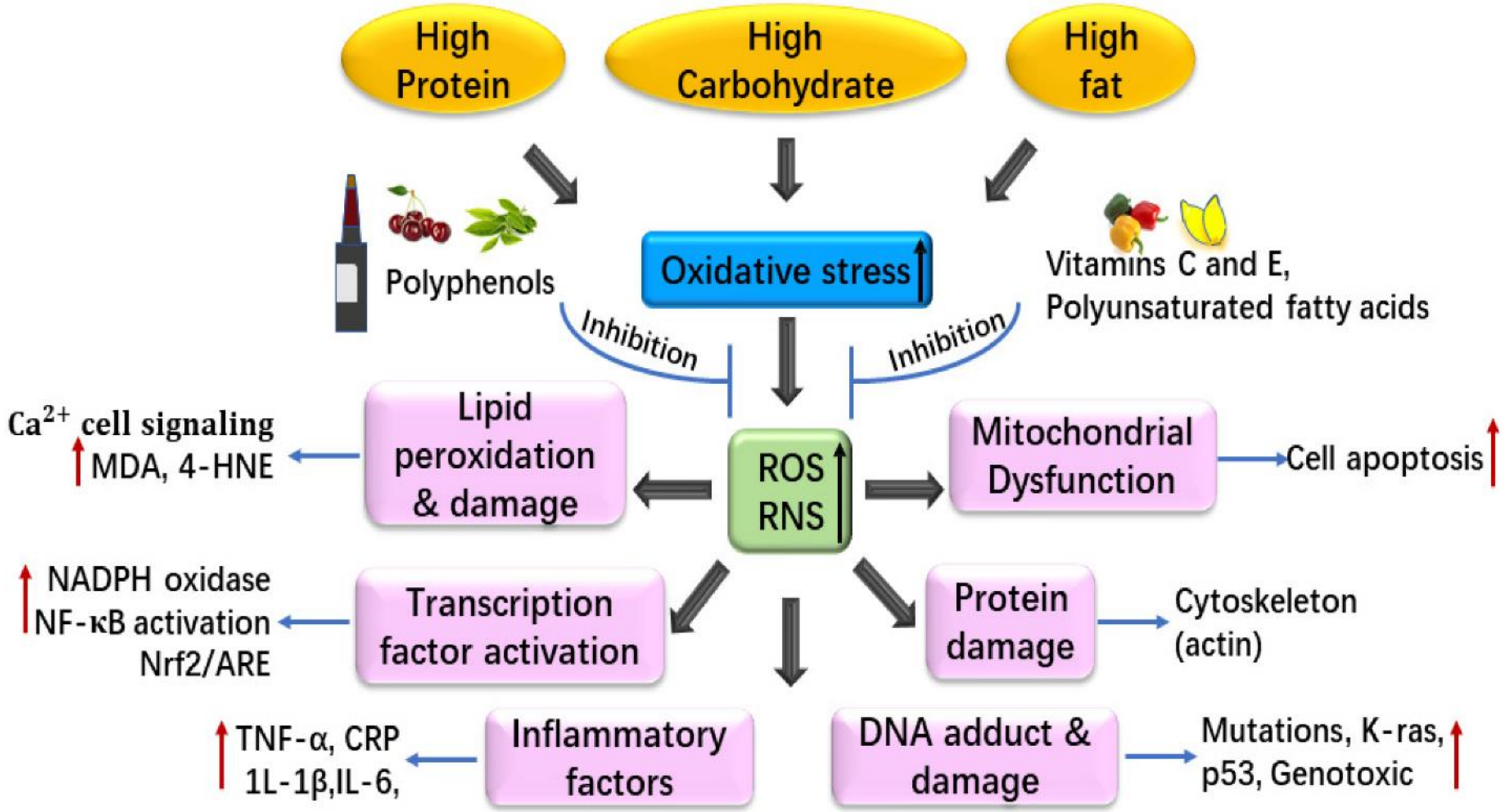
➢This imbalance between pro-oxidants and antioxidants can lead to a number of reproductive diseases such as endometriosis, polycystic ovary syndrome (PCOS), and unexplained infertility.

Oxidative stress

- Pregnancy complications such as spontaneous abortion, recurrent pregnancy loss, preeclampsia, andintrauterine growth restriction (IUGR) can also develop in response to OS
- OS in female reproduction is suggested to affect follicle and oocyte maturation, ovarian steroidogenesis and embryo and placenta development .
- The impact of OS on assisted reproductive techniques (ART)

The proposed mechanism

- The proposed mechanism is the effect of elevated ROS on cellular biomolecules (lipids, proteins and nucleic acids) and other cellular materials which lead to biomolecular damage such as lipid peroxidation of the cell membrane, deoxyribonucleic acid damage, inhibition of protein synthesis, depletion of adenosine tri-phosphate and cell apoptosis.
- The most frequently used methods with high sensitivity and high reproducible results in assessing biomolecular damage measure malondialdehyde (MDA) and total antioxidant capacity (T-AOC)


High Protein
High Carbohydrate
High fat
Polyphenols
Oxidative stress
Vitamins C and E,
Polyunsaturated fatty acids
Inhibition
Inhibition
Ca2+ cell signaling
MDA, 4-HNE
Lipid peroxidation & damage
ROS RNS
Mitochondrial Dysfunction
Cell apoptosis
NADPH oxidase
NF-κB activation
Nrf2/ARE
Transcription factor activation
Protein damage
Cytoskeleton (actin)
TNF-α, CRP
1L-1β,IL-6,
Inflammatory factors
DNA adduct & damage
Mutations, K-ras, p53, Genotoxic

Oxidative stress in female reproduction

- Each month, a cohort of oocytes begin to grow and develop in the ovary, but meiosis I resumes in only one of them, the dominant oocyte. This process is targeted by an increase in ROS and inhibited by antioxidants.
- In contrast, the progression of meiosis II is promoted by antioxidants, suggesting that there is a complex relationship between ROS and antioxidants in the ovary.

Age-related fertility decline and menopause

- Aging is defined as the gradual loss of organ and tissue functions. Oocyte quality decreases in relation to increasing maternal age. Recent studies have shown that low quality oocytes contain increased mtDNA damage and chromosomal aneuploidy, secondary to age-related dysfunctions. These mitochondrial changes may arise from excessive ROS.
- Menopause also leads to a decrease in estrogen and the loss of its protective effects against oxidative damage to the endometrium.

Endometriosis

- Endometriosis is a benign, estrogen-dependent, chronic gynecological disorder characterized by the presence of endometrial tissue outside the uterus.
- It is evident that endometriotic cells contain high levels of ROS; however, their precise origins remain unclear. Impaired detoxification processes lead to excess ROS and OS, and may be involved in increased cellular proliferation and inhibition of apoptosis in endometriotic cells.
- Further studies investigating dietary and supplemental antioxidant intake within different populations are warranted to determine if antioxidant status and/or intake play a role in the development, progression, or regression of endometriosis.

Polycystic ovary syndrome

- Polycystic ovary syndrome is the most common endocrine abnormality of reproductive-aged women and has a prevalence of approximately 18%.
- Polycystic ovary syndrome is also associated with decreased antioxidant concentrations, and is thus considered an oxidative state. The decrease in mitochondrial O_2 consumption and GSH levels along with increased ROS production explains the mitochondrial dysfunction in PCOS patients.
- Physiological hyperglycemia generates increased levels of ROS from mononuclear cells, which then activate the release of TNF-alpha, a known mediator of insulin resistance, are further increased. The resultant OS creates an inflammatory environment that further increases insulin resistance and contributes to hyperandrogenism .

Polycystic ovary syndrome

- Lifestyle modification is the cornerstone treatment for women with PCOS.
- This includes exercise and a balanced diet, with a focus on caloric restriction .
- However, if lifestyle modifications do not suffice, a variety of options for medical therapy exist.
- Combined oral contraceptives are considered the primary treatment for menstrual disorders.
- Currently, there is no clear primary treatment for hirsutism, although it is known that combination therapies seem to produce better results .

Spontaneous abortion &Recurrent pregnancy loss

- Between 10 and 12 weeks of gestation, the trophoblastic plugs are dislodged from the maternal spiral arteries, flooding the intervillous space with maternal blood.
- This event is accompanied by a sharp rise in O_2 tension, marking the establishment of full maternal arterial circulation to the placenta associated with an increase in ROS, which leads to OS .
- At physiological concentrations, ROS stimulate cell proliferation and gene expression .

Spontaneous abortion &Recurrent pregnancy loss

- Placental acclimation to increased O_2 tension and OS at the end of the 1^{st} trimester up-regulates antioxidant gene expression and activity to protect fetal tissue against the deleterious effects of ROS during the critical phases of embryogenesis and organogenesis.
- Amongst the recognized placental antioxidants are heme oxygenase (HO)-1 and -2, Cu,Zn-SOD, catalase, and GPx .
- If maternal blood flow reaches the intervillous space prematurely, placental OS can ensue too early and cause deterioration of the syncytiotrophoblast. This may give rise to a variety of complications including miscarriage, recurrent pregnancy loss, and preeclampsia, amongst others. These complications will be discussed below.

Preeclampsia

- Preeclampsia is a complex multisystem disorder that can affect previously normotensive women.
- The major pathophysiologic disturbances are focal vasospasm and a porous vascular tree that transfers fluid from the intravascular to the extravascular space.
- The exact mechanism of vasospasm is unclear, but research has shown that interactions between vasodilators and vasoconstrictors, such as NO, endothelin 1, angiotensin II, prostacyclin, and thromboxane, can cause decrease the perfusion of certain organs.
- The porous vascular tree is one of decreased colloid osmotic pressure and increased vascular permeability

Preeclampsia

- Placental ischemia/hypoxia is considered to play an important role through the induction of OS, which can lead to endothelial cell dysfunction and systemic vasoconstriction. From early pregnancy on, the body assumes a state of OS.
- Oxidative stress is important for normal physiological functions and for placental development. Preeclampsia, however, represents a much higher state of OS than normal pregnancies.
- Affected women also have a decreased total antioxidant status (TAS), placental GPx, and low levels of vitamins C and E.
- Inadequate vitamin C intake seems to be associated with an increased risk of preeclampsia.

Preterm labor

- Preterm labor occurs before 37 weeks of gestation and is the leading cause of perinatal morbidity and mortality worldwide
- Women with preterm labor have lower levels of TAS than women with uncomplicated pregnancies at a similar gestational age, which might indicate the presence of increased OS during preterm labor.
- Women with preterm births have also been found to have significantly decreased PON 1 activity in comparison to controls. This finding suggests that enhanced lipid peroxidation and diminished antioxidant activity of PON 1, may together create a pro-oxidant setting and increase the risk for preterm birth.

Paraoxonase-1 (PON 1), an enzyme associated with HDL, acts to offset LDL oxidation and prevent lipid peroxidation

Preterm labor

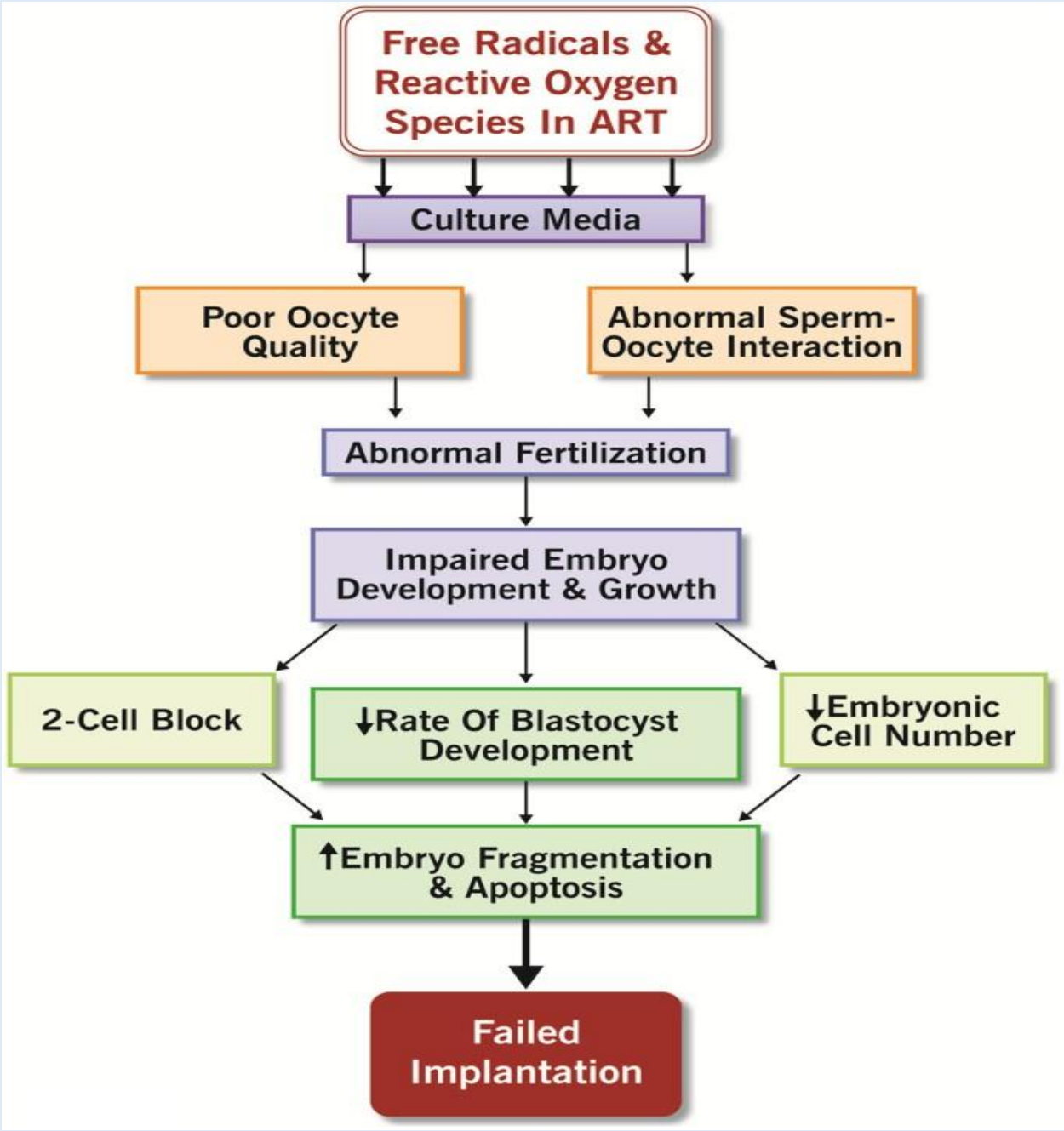
- Additionally, patients in preterm labor had markedly decreased levels of GSH . Low maternal serum selenium levels in early gestation have been associated with preterm birth
- Polymorphism to GST was found to be significantly higher in patients in preterm labor, indicating that these patients are more vulnerable to oxidative damage .
- The inflammatory setting of maternal infection associated with preterm birth produces a state of OS and the consequent decrease in antioxidant defenses are likely to increase the risk for preterm birth.

Preterm labor

- The presented evidence implicates inflammation and suppressed antioxidant defenses in the pathogenesis of preterm labor. Thus, it seems plausible that antioxidant supplementation may assist in preventing preterm labor and birth associated with inflammation.

Intrauterine growth restriction

- Intra uterine growth restriction is defined as infant birth weight below the 10^{th} percentile. This condition affects 10% of newborns.
- Studies also indicate that patients with IUGR develop OS because of placental ischemia/reperfusion injury secondary to improper spiral arteriole development.
- Imbalanced injury and repair as well as abnormal development of the villous tree are characteristic of IUGR placentas, predisposing them to depletion of the syncytiotrophoblast with consequently limited regulation of transport and secretory function. As such, OS is recognized as an important player in the development of IUGR.
- Ischemia and reperfusion injury are powerful generators of ROS and OS.


Free Radicals & Reactive Oxygen Species In ART
Culture Media
Poor Oocyte Quality
Abnormal Sperm-Oocyte Interaction
Abnormal Fertilization
Impaired Embryo Development & Growth
2-Cell Block
↓Rate Of Blastocyst Development
↓Embryonic Cell Number
↑Embryo Fragmentation & Apoptosis
Failed Implantation

How we reduce oxidative stress

- Avoiding exposure to unnieciary oxidation and increasing antioxidants.

Antioxidants

- Antioxidants are scavengers that detoxify excess ROS, which helps maintain the body's delicate oxidant/antioxidant balance.

  There are two types of antioxidants:

  ***enzymatic* and *non-enzymatic*.**

Enzymatic antioxidants

- Enzymatic antioxidants possess a metallic center, which gives them the ability to take on different valences as they transfer electrons to balance molecules for the detoxification process.
- They neutralize excess ROS and prevent damage to cell structures.
- Endogenous antioxidants enzymes include SOD, catalase, GPx, and glutathione oxidase.
- Dismutation of the SO anion to H_2O_2 by SOD is fundamental to anti-oxidative reactions. The enzyme SOD exists as three isoenzymes. They contain Cu and zinc or manganese as metal co-factors

Enzymatic antioxidants

- The glutathione (GSH) family of enzymes includes GPx, GST, and GSH reductase. GPx uses the reduced form of GSH as an H^+ donor to degrade peroxides. Depletion of GSH results in DNA damage and increased H_2O_2 concentrations; as such, GSH is an essential antioxidant.
- Glutathione peroxidase exists as five isoforms in the body: GPx1, GPx2, GPx3, GPx4, and GPx5.

glutathione reductase (GR), **glutathione** peroxidase (**GPx**), and **glutathione**-S-transferase (**GST**)

Non-enzymatic antioxidants

- The non-enzymatic antioxidants consist of dietary supplements and synthetic antioxidants such as vitamin C, GSH, taurine, hypotaurine, vitamin E, Zn, selenium (Se), beta-carotene, and carotene.
- Vitamin C (ascorbic acid) is a known redox catalyst that can reduce and neutralize ROS.
- Glutathione is a peptide found in most forms of aerobic life as it is made in the cytosol from cysteine, glutamate, and glycine; it is also the major non-enzymatic antioxidant found in oocytes and embryos.

Glutathione exists in reduced (GSH) and oxidized (GSSG) states. The ratio of reduced glutathione to oxidized glutathione within cells is a measure of cellular oxidative stress[

Non-enzymatic antioxidants

- Taurine and hypotaurine are scavengers that help maintain redox homeostasis in gametes. Both neutralize lipid peroxidation products, and hypotaurine further neutralizes hydroxyl radicals.
- Vitamin E (α-tocopherol) is a lipid soluble vitamin with antioxidant activity. It consists of eight tocopherols and tocotrienols. It plays a major role in antioxidant activities because it reacts with lipid radicals produced during lipid peroxidation.

Non-enzymatic antioxidants

- The hormone melatonin is an antioxidant that, unlike vitamins C and E and GSH, is produced by the human body. In contrast to other antioxidants, however, melatonin cannot undergo redox cycling; once it is oxidized, melatonin is unable to return to its reduced state because it forms stable end-products after the reaction occurs.
- Transferrin and ferritin, both iron-binding proteins, play a role in antioxidant defense by preventing the catalyzation of free radicals through chelation .
- Nutrients such as Se, Cu, and Zn are required for the activity of some antioxidant enzymes, although they have no antioxidant action themselves.

Antioxidants

- Antioxidant supplementation may be effective in controlling the production of ROS and continues to be explored as a potential strategy to overcome reproductive disorders associated with infertility. However, investigations conducted to date have been through animal or in vitro studies, which have produced largely conflicting results.

- **Studies have shown that extremes of body weight and lifestyle factors such as cigarette smoking, alcohol use, and recreational drug use can promote excess free radical production, which could affect fertility. Exposures to environmental pollutants are of increasing concern, as they too have been found to trigger oxidative states, possibly contributing to female infertility.**

Lifestyle factors

Obesity/overnutrition

- Oxidative stress from excessive ROS generation has been implicated in pathogenesis of obesity.
- Intracellular fat accumulation can disrupt mitochondrial function, causing buildup and subsequent leak of electrons from the ETC. The combined effect of high lipid levels and OS stimulates production of oxidized lipids; of particular importance are lipid peroxides, oxidized lipoproteins, and oxysterols.
- As major energy producers for cells, the mitochondria synthesize ATP via oxidative phosphorylation. Adverse effects of maternal BMI on mitochondria in the oocyte could negatively influence embryonic metabolism.

Lifestyle factors

Malnutrition/underweight

- In-utero undernutrition reduces NO stores, triggering OS along with impairment of endothelium-dependent vasodilation.

Exercise

- Physical exercise produces an oxidative state due to excessive ROS generation. Any type of extreme aerobic or anaerobic activity (e.g. marathon running, weight training) may contribute to cellular damage. Optimal amounts of OS are necessary for physiologic functioning.
- Physical activity causes an increase in ROS, which in turn heightens antioxidant response, thus providing protection from future attacks.

Lifestyle factors

- **Cigarette smoking**
- **Alcohol use**
- **Recreational drug use:**

  Cannabinoids: Cannabinoids are active constituents of marijuana

  Cocaine
- **Environmental and occupational exposures**
- Organochlorine pesticides: DDT
- Polychlorinated biphenyls
- Organophosphate pesticides

- An overproduction of OS after acute exercise in certain diseased individuals may serve as a trigger for improved antioxidant defense when compared with their healthy counterparts

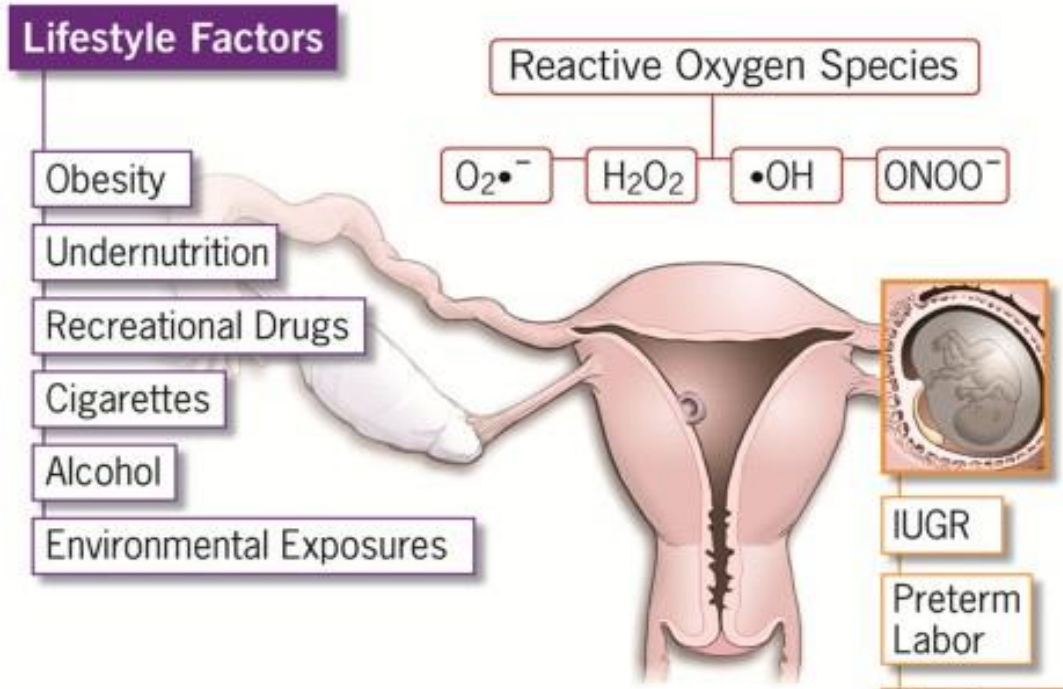
Oxidative Stress

Lifestyle Factors

- Obesity
- Undernutrition
- Recreational Drugs
- Cigarettes
- Alcohol
- Environmental Exposures

Reactive Oxygen Species

$O_2\bullet^-$ | H_2O_2 | $\bullet OH$ | $ONOO^-$



- IUGR
- Preterm Labor
- Spontaneous Abortion

Reproductive Pathology and Physiology

- PCOS
- Endometriosis
- Preeclampsia
- Age-related Fertility Decline & Menopause
- Unexplained Infertility

↓

Infertility


Thanks for your
attention