# Oxidative stress, mitochondria, and infertility: is the relationship fully established?

Oxygen is thought to have been formed in the universe when stars much larger than our own sun were born, burning their stored hydrogen and creating heavier chemical elements by nuclear fusion reactions. Explosions of these giant dying stars scattered all chemical elements into space as stardust, which became the base material for the formation of our solar system. Nevertheless, diatomic oxygen, or free oxygen, which now makes up 20.9% of our atmosphere, appeared much later than the earth itself. In fact, when life was evolving 2.4 billion years ago, cyanobacteria able to use sunlight to produce energy in the form of carbohydrate and oxygen as a waste product of such chemical processes emerged. Oxygen levels increased over time, resulting in the development of new bacteria with more efficient oxygen-dependent energy-producing systems. These prokaryotic bacteria, known as mitochondria ancestors, were incorporated into larger host cells to serve as engines for energy supply. The payoff of this successful endosymbiosis approximately 1.5 billion years ago was the appearance of the aerobic eukaryotic cell, which further evolved into multicellular organisms, leading to the development of specialized organs for oxygen intake, distribution, and processing to supply the body's constant metabolic need for energy production in all cell compartments.

The mitochondria in our cells are highly efficient engines in the form of organelles that specialize in energy production in the presence of oxygen. This process, known as oxidative phosphorylation, occurs through a series of electron transfers and redox reactions, catalyzing the protein complexes in the mitochondrial membranes. Processing oxygen is complex, because it generates reactive oxygen species (ROS) like superoxide and hydrogen peroxide, which are unstable and potentially toxic to cells. However, mitochondrial function not only creates the problem of ROS accumulation but in addition contributes to solving it by activation of a number of antioxidant defense systems.

Antioxidant response is coordinated to adapt to ROS levels. Indeed, at low levels, ROS act as signaling molecules related to numerous pathways involved in cell proliferation, differentiation, and survival. Conversely, at high levels, ROS can damage deoxyribonucleic acid (DNA), lipids, and proteins in cells, activating cell renewal mechanisms like autophagy or cell death pathways like apoptosis. In the ovary, ROS play a major role as signaling molecules, not only for physiological follicle atresia through activation of apoptosis pathways, but also for follicle development and oocyte maturation. However, the antioxidant activity required to balance ROS levels is complex in follicle development, because along with the acute increase in ROS levels that sustains rapid granulosa cell growth, a significant upturn in antioxidant activity is additionally needed to protect the final maturation of oocytes. Mature oocytes are the largest mammalian cells, having to provide enough substrate for embryo development up to implantation in the endometrium. Rapid oocyte growth requires an efficient engine, which is made available by the presence of a large mitochondrial mass and abundance of substrates like glucose and fatty acids for oxidative phosphorylation. Indeed, among eukaryotic cells with heterogeneous mitochondrial DNA (mtDNA) copy numbers range from 1,000 to 200,000 copies, and oocytes reach around 150,000 mtDNA copies, exhibiting a 30-times increase during their maturation to supply good-quality energy substrate for embryo development.

Unbalanced ROS levels because of mitochondrial abnormalities causing greater ROS generation, a fall in antioxidant capacity, or the presence of a chronic proinflammatory environment may increase the risk of infertility. Indeed, oxidative stress has a detrimental impact, because it is associated with the accumulation of DNA mutations, genomic instability, accelerated aging, and progression of pathological conditions like chronic inflammation and cancer development.

## ROLE OF AGE

Advanced maternal age is known to be related to a decrease in the size and quality of the ovarian reserve, resulting in fertility decline. However, perceptions of ovarian aging and their implications have changed in recent years. On one hand, specific mechanisms against cell senescence play a crucial role in keeping immature oocytes undisturbed and virtually immortalized throughout the first decades of a woman's life, until they are selected to complete meiotic division and become potentially fertilizable. On the other hand, ovarian aging and diminishing activity anticipates general aging, as life expectancy has increased significantly over the last century, whereas the mean age of menopause has never changed. Moreover, women's rightful aspirations for education, career building, and exploration of other life experiences before or instead of motherhood have led to postponement of childbearing to later years. Delaying pregnancy to more advanced reproductive age increases the number of women experiencing difficulties conceiving their own genetic offspring.

Although efficient, maintenance of oocyte quiescence and quality undergoes age-related decline involving various anomalies, including defects in the meiotic machinery, like abnormal chromosomal segregation engendering a higher risk of aneuploidy, and mitochondrial dysfunction, namely decreased mtDNA copy numbers and alterations to morphology and function, with a reduced energy supply and greater oxidative stress-related damage. Therapeutic strategies targeting mitochondrial function have been mostly investigated in various aging models. The rationale is to provide necessary substrates or enhance activity in mitochondrial antioxidant systems to protect oocytes from oxidative stress. Although some antioxidants are potential clinical candidates to boost oocyte quality in age-related fertility decline, the causal relationship between oxidative stress and oocyte competence is still unclear in the course of each meiotic step (1).

## OXIDATIVE STRESS AND ENDOMETRIOSIS

Endometriosis is a chronic disease of reproductive age, whose main symptoms of pelvic pain and infertility are

related to chronic inflammation. The latter is especially interconnected with oxidative stress, as triggering of inflammation contributes to increased ROS generation, and ROS in turn act as signaling molecules to enhance proinflammatory gene expression. The updated redefinition of endometriosis as a systemic disease affecting not only the pelvic cavity but also cardiovascular, neurological, immune, and metabolic functions underlines how important the role of chronic inflammation is in this pathological condition. Moreover, the average delay until diagnosis, ranging from 4 to 11 years, may further contribute to the severe fertility decline that up to 50% of patients with endometriosis experience (2). It is therefore unsurprising that inflammation and oxidative stress markers are usually found to be increased in women with endometriosis, because numerous sources of ROS are related to its pathogenesis and progression, including greater lysis of erythrocytes, with subsequent iron overload and apoptotic endometrial tissue inducing abnormal macrophage activation and leading to chronic inflammation (3). In addition, numerous pathogenic mechanisms have been explored to explain endometriosis-related infertility, such as pelvic exposure to a toxic environment, anatomical dysfunction of the fallopian tubes and/or ovary, alterations to endometrial receptivity, and impairment of follicle development and oocyte maturation. Complete transcriptomic profiling of oocytes from women affected by endometriomas and non-ovarian endometriosis compared with that of unaffected women has shed light on differences in gene expression patterns (4). Oocytes from subjects with endometriosis showed increased function in metabolism and biosynthesis of lipids and steroids and in their oxidative stress response, irrespective of the presence of ovarian endometriomas, reinforcing the hypothesis of a systemic pathological condition. This study raised new questions about the link between oxidative stress and infertility in endometriosis, suggesting that alterations in antioxidant capacity may be responsible for poor oocyte quality. However, cause-and-effect relationships between oxidative stress, chronic inflammation and infertility in endometriosis require further investigation.

## MITOCHONDRIAL FUNCTION IN POLYCYSTIC OVARY SYNDROME

Polycystic ovary syndrome (PCOS) is an endocrine and metabolic disorder characterized by hyperandrogenism, chronic ovulation, and alterations in both lipid and glucose metabolism. In the light of this already complex setting, the chronic inflammatory environment and increased ROS levels play a major role and appear to be positively correlated with more severe progression towards insulin resistance, diabetes, and cardiovascular disorders.

Chronic anovulation in infertile patients with PCOS clearly reduces the chances of pregnancy and, although it can be successfully managed by controlled ovarian stimulation, data on oocyte maturation and quality are still contentious and need more scrutiny. Recent hypotheses suggested preexisting mitochondrial dysfunctions in the pathogenesis of PCOS, including impairment of bioenergetic supply and imbalance in ROS generation (5). Defective mitochondria may not only trigger metabolic changes in favor of dyslipidemia and insulin resistance but also contribute to impaired ovulation and oocyte quality. Genetic and epigenetic mitochondrial mutations have been evidenced in oocytes from patients with PCOS, as have abnormal mtDNA copy numbers. The latter were found to be decreased in PCOS oocytes during their maturation in some studies, but others observed an increase in mtDNA copy numbers as well as antioxidant activity as a compensating mechanism to address preexisting defective mitochondrial function (5). The discrepancy in these findings may be explained by different disease stages and phenotypes. Nevertheless, although the imbalance in ROS and antioxidant capacity is already well documented in PCOS, further investigations are warranted to assess the role of oxidative stress in the pathogenesis of PCOS-related infertility and impaired oocyte maturation.

In conclusion, there is increasing evidence of a relationship between oxidative stress and female fertility decline when examining both age-related oocyte quality decline and pathological conditions related to chronic inflammation. The key component appears to be the mitochondria, whose mass and function are closely connected to favorable oocyte and embryo outcomes by supplying sufficient energy and balancing ROS generation in the process. However, the specifics of this relationship are still unclear, because oxidative stress may be the trigger for biological dysfunction, leading to poor fertility results, or an epiphenomenon occurring as a consequence of compromised cell function. Shedding light on the mechanisms by which oxidative stress is involved in the pathogenesis of infertility may not only yield valuable fundamental insights into human ovarian physiology and pathology but also aid in development of new therapeutic strategies to enhance fertility outcomes in women.

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