

New Insights in Molecular Mechanisms of Male Infertility

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A better knowledge of the cellular and molecular mechanisms involved in the origin of many male-factor infertility situations is crucial to establish appropriate therapeutic strategies. This article reviews the role of reactive oxygen species (ROS) and male-factor infertility, oxidative stress as a marker of DNA damage and apoptosis and spermatozoa.

Reactive Oxygen Species and Male-Factor Infertility

A small amount of ROS, highly reactive oxidizing agents that belong to a class of free radicals, is necessary for spermatozoa to acquire fertilizing abilities. Seminal oxidative stress develops when ROS production overwhelms scavenging activities. High levels of ROS are detected in semen samples of 25% to 40% of infertile men. Spermatozoa are particularly susceptible to ROS damage by lipid peroxidation because their plasma membranes contain large quantities of polyunsaturated fatty acids and low concentrations of scavenging enzymes.

Various studies have suggested that a defect in the regulation of normal spermatogenesis leads to increased production of immature sperm with cytoplasmic retention that produces high levels of ROS. Recent studies have shown that high levels of ROS production in semen are negatively correlated with the percentage of normal sperm forms. Abnormal spermatozoa and leukocytes can produce high amounts of ROS. Levels of ROS in patients with no seminal leukocytes are re-

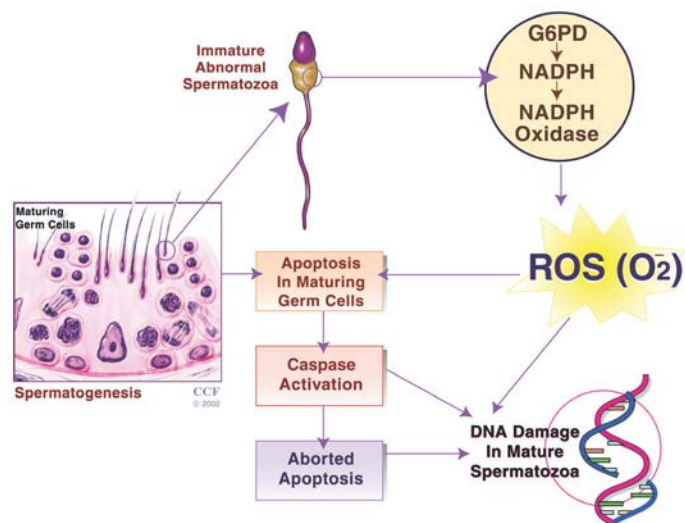
portedly lower than levels in patients with WHO-defined leukocytospermia. Poor sperm quality in such infertile patients may be due to leukocyte-mediated oxidative stress resulting in DNA damage, which may be related to alterations in the regulation of spermatogenesis.

High levels of ROS production have been found in patients with male-factor infertility and various clinical diagnoses such as spinal cord injury, clinical varicocele, vasectomy reversal, chronic prostatitis, and genitourinary tract infections and in men with a history of smoking. Normospermic infertile patients also have higher levels of ROS than controls. A close relationship has been observed between ROS production and the stage of germ cell development, with ROS production being highest in immature spermatozoa.

Both ROS and total antioxidant capacity by themselves are not efficient discriminators of oxidative stress. A novel composite ROS-total antioxidant capacity (ROS-TAC) score derived using principal component analysis has been shown to be an important marker of oxidative stress and DNA damage. The ROS-TAC score has been found to be significantly reduced in infertile patients who failed to initiate pregnancy vs. those who were successful and negatively correlated with sperm DNA damage.

Oxidative Stress as a Marker of DNA Damage

ROS attacks the fluidity of the sperm plasma membrane and the integrity of DNA in the



Possible mechanism of DNA damage in human spermatozoa

sperm nucleus. Oxidative stress has been correlated with high frequencies of single- and double-strand DNA breaks. Strong evidence suggests that high levels of ROS mediate the DNA fragmentation that is commonly observed in the spermatozoa of infertile men.

The extent of DNA damage in infertile men with no detectable abnormalities in any of the standard semen parameters was negatively correlated with fertilization rate, embryo quality and overall pregnancy. Pregnancy failure following assisted reproduction may be related, at least in part, to increased DNA damage in spermatozoa.

Apoptosis and Spermatozoa

Apoptosis (programmed cell death) is a regulated physiological process that leads to cell shrinkage, membrane blebbing, and DNA fragmentation. In somatic cells, mitochondria appear to be the central regulators of apoptosis. Caspases, a family of cysteine aspartic acid proteases, are central regulators of apoptosis. Apoptosis plays an important role in regulating

spermatogenesis of various mammalian species, including humans. Apoptosis has been extensively investigated in spermatogonia, spermatocytes and spermatids in the testis, and many apoptotic factors have been identified.

Conflicting data have been reported on apoptosis in ejaculated sperm, and the biological significance of apoptosis in ejaculated spermatozoa is unclear. The physiological presence of apoptosis has been demonstrated in human spermatogenic epithelium indicating that the process is necessary for normal function of the adult reproductive tissues. Some forms of male infertility may result from an abortive program of germ cell death. In this case, the abortive process is incomplete and fails to eliminate germ cells even though the apoptotic programs have been initiated. These rescued cells undergo spermiogenesis and spermatogenesis resulting in mature sperm with an apoptotic phenotype often found in human semen from infertile patients.