

## Nitric oxide and human sperm motility: an immunohistochemical study

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Defective sperm function is a common cause of human male infertility.

Human spermatozoa are very susceptible to oxidative stress, probably because they contain high levels of polyunsaturated fatty acids and are exposed to ROS produced by contaminating neutrophils or by the spermatozoa themselves. In addition, due to the cytoplasm scarcity, they lack an adequate reserve of antioxidant enzymes.

It has been previously shown that human spermatozoa produce nitric oxide (NO) as well as superoxide anion and consequently peroxynitrite, which can rapidly react with proteins, lipids, and nucleic acids.

NO is a short-lived free radical produced by three isoforms of NO synthase (NOS): endothelial (eNOS), neuronal (nNOS), and inducible (iNOS), which are responsible for the conversion of L-arginine to L-citrulline and NO. NO plays an important role in sperm physiology, although at high concentrations it could lead to cytotoxicity accompanied by decreased sperm motility.

In recent studies it has been evidenced the presence of a significant negative linear correlation between NO concentration and sperm motility, as well as between peroxynitrite production and the kinetic features of spermatozoa.

It has been previously demonstrated that the spermatozoa of fertile men express both eNOS and nNOS, which appear to be involved in sperm motility, metabolism, capacitation and acrosome reaction. However, the specific role of NOS isoforms in gametes is not well understood, even if iNOS seems to negatively affect the sperm function through the production of large amounts of NO, and consequently the nitration of protein tyrosine by peroxynitrite.

The aim of the present study was to assess the presence and localization of NOS isoforms in human spermatozoa isolated from controls and asthenozoospermic infertile patients, by means of immunohistochemical techniques, in order to characterize the pattern of NOS expression and to evaluate the correlation with sperm function. In addition we studied the immunoeexpression of citrulline, to achieve the microscopic visualization of NOS catalytic activity, and nitrotyrosine, since the protein tyrosine nitration represents a shift from the physiological role of NO toward pathogenetic pathways.

The main results of our study are that the immunohistochemical expression of constitutive NOS isoforms is lower in the asthenozoospermic group, while the immunoeexpression of iNOS and nitrotyrosine is higher in comparison with normozoospermic fertile subjects, thus suggesting a possible pathogenetic role of NO in the reduction of sperm motility.

Keywords: spermatozoa, asthenozoospermia, nitric oxide synthase, citrulline, nitrotyrosine, immunohistochemistry