

## Assessment of reactive oxygen species production in semen by the nitroblue tetrazolium reduction assay (NBT)

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**Introduction:** The presence of high reactive oxygen species (ROS) levels in semen is a major factor involved in the decline of male fertility by impairing sperm motility and DNA integrity. In semen, ROS are mainly produced by leucocytes and abnormal sperm. We aimed to assess *in situ* the ROS production in spermatozoa and leucocytes in the ejaculate of infertile men using photometric nitroblue tetrazolium (NBT) reduction assay.

**Material & Methods:** 38 semen samples from infertile patients were investigated by semen analysis and stained for ROS production with nitro blue tetrazolium. The measurement of spermatozoa and leucocytes ROS generation was evaluated via production of coloured formazan.

**Results:** The mean value of positive staining NBT (NBT+) sperm was 9.4% (2-35%). NBT (+) sperm rates were significantly higher in the teratospermic group in comparison with the

normal morphology sperm group. We noted positive and significant correlations between the levels of (NBT+) staining sperm and some sperm morphological abnormalities: cytoplasmic droplets, flagellar angulation and coiled tails. Moreover, leucospermia was found in 28.9% of our samples and we noted a significant negative correlation between (NBT+) staining leucocytes in semen and typical morphology spermatozoa rates.

**Conclusion:** The NBT test is an inexpensive and easy-to-perform assay that can be routinely applied to identify sperm oxidative stress in infertile men. This assay can be used for the evaluation of seminal leucocytes activation state. It can also have significant clinical utility by the exploration of some unexplained infertility and *in vitro* fertilization failures in infertile couples.

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