**NO level and oxidative stress in viable spermatozoa of infertile males**

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**Introduction.** Nitric oxide (NO) has been shown to be important in sperm function, and an overproduction of NO plays a negative effect on semen parameters. Moreover, sperm membrane contains high concentrations of polyunsaturated fatty acids that are highly susceptible to oxidative damage. Under pathological conditions, the production of reactive oxygen/nitrogen species (ROS/RNS) often increases, resulting in oxidative stress that can exert negative effects against spermatozoa and male reproductive system (1).

**Aim.** The purpose of this study is: 1. to investigate the levels of malondialdehyde (MDA) a marker of lipid peroxidation, NO and the antioxidants ascorbic acid (AA), reduced (GSH) and oxidized (GSSG) glutathione in viable spermatozoa of men; 2. to establish a relationship between the oxidative stress parameters and the sperm features; 3. to explore if viable sperm cells themselves exhibit a control of oxidative stress comparing a group of infertile men to a control group.

**Methods.** From June 2015 through October 2016, we enrolled 93 cases (aged 24-47 years) following the inclusion criteria: non azoospermic men with a normal karyotype. The subjects were grouped in patients with fertility problems (group 1) and men who wanted to be tested for fertility (group 2). Sperm viability was determined and sperm cells were suspended in 1 ml of PBS and lysed through rapid freeze-thawing at -80 oC and +35 oC, for three times respectively. Successively, the samples were centrifuged at 2500 g for 5 minutes and were divided into aliquots stored at -80 oC until analysis. NO levels were determined with colorimetric Kit (Cayman), GSH and GSSG were measured spectrophotometrically and MDA was assayed with an HPLC method.

**Results.** The analysis of data in 93 individuals showed NO level was negatively correlated with sperm concentration ($P<0.001$), motility ($P<0.05$), sperm with normal morphology ($P<0.001$) and sperm viability ($P<0.05$). NO concentration was positively correlated with MDA and AA levels ($P<0.001$), GSH and GSSG levels ($P<0.001$). AA level was negatively correlated with sperm parameters (sperm concentration and sperm with normal morphology: $P<0.001$); sperm motility: $P<0.05$; sperm viability: $P<0.01$) and positively correlated with GSH and GSSG concentrations ($P<0.001$). When group 1 was compared to group 2, sperm concentrations ($P<0.05$), sperm motility ($P<0.001$), sperm with normal morphology ($P<0.05$), sperm viability ($P<0.001$) were significantly decreased in group 1 (fertility problems) respect to those observed in group 2. NO ($P<0.001$), AA ($P<0.05$) GSH ($P<0.01$) levels were significantly increased, whereas GSSG level ($P<0.01$) were decreased in group 1 respect to group 2. MDA concentration was similar in the two groups.

**Conclusion**. In our work, the behavior of AA and GSH in viable spermatozoa of infertile group, seems to express an higher capacity of defense against the oxidative damage respect to control group. We speculate that increasing GSH and AA, help to counteract the formation of ROS/RNS by

preventing the LPO in viable sperm of infertile group. This finding is encouraging in view the use of the sperm of infertile patients for Intracytoplasmatic Sperm Injection (ICSI) technique.

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