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TiO_2 Nanoparticles Induce DNA Damage and Expression of Biomarker of Oxidative Stress on Human Spermatozoa

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Abstract : The increasing production and the use of TiO_2 nanoparticles (NPs) have inevitably led to their release into the environment, thereby posing a threat to organisms and also for human. Human exposure to TiO_2-NPs may occur during both manufacturing and use. TiO_2-NPs are common in consumer products for dermal application, toothpaste, food colorants, and nutritional supplements, then oral exposure may occur during use of such products. Into the body, TiO_2-NPs thanks to their small size (<100 nm), can, through testicular blood barrier inducing effect on testis and then on male reproductive health. The nanoscale size of TiO_2 increase the surface-to-volume ratio making them more reactive in a cell, then TiO_2 NPs increase their ability to produce reactive oxygen species (ROS). In male germ cells, ROS may have important implications in maintaining the normal functions of mature spermatozoa at physiological levels, moreover, in spermatozoa they are important signaling molecules for their hyperactivation and acrosome reaction. Nevertheless, an excess of ROS by external inputs such as NPs can increased the oxidative stress (OS), which results in damage DNA and apoptosis. The aim of our study has been investigate the impact of TiO_2 NPs on human spermatozoa, evaluating DNA damage and the expression of proteins involved in cell stress. According WHO guidelines 2021, we have exposed human spermatozoa in vitro to TiO_2 NP at concentrations 50 ppm, 100 ppm, 250 ppm, and 500 ppm for 1 hour (at 37°C and CO_2 at 5%). DNA damage was evaluated by Sperm Chromatin Dispersion Test (SCD) and TUNEL assay; moreover, we have evaluated the expression of biomarkers of oxidative stress like Heat Shock Protein 70 (HSP70) and Metallothioneins (MTs). Also, sperm parameters as motility viability have been evaluated. Our results not report a significant reduction in motility of spermatozoa at the end of the exposure. On the contrary, the progressive motility was increased at the highest concentration (500 ppm) and was statistically significant compared to control ($p < 0.05$). Also, viability was not changed by exposure to TiO_2-NPs ($p < 0.05$). However, increased DNA damage was observed at all concentrations, and the TUNEL assay highlighted the presence of single strand breaks in the DNA. The spermatozoa responded to the presence of TiO_2-NPs with the expression of Hsp70, which have a protective function because they allow the maintenance of cellular homeostasis in stressful/ lethal conditions. A positivity for MTs was observed mainly for the concentration of 4 mg/L. Although the biological and physiological function of the metallothionein (MTs) in the male genital organs is unclear, our results highlighted that the MTs expressed by spermatozoa maintain their biological role of detoxification from metals. Our results can give additional information to the data in the literature on the toxicity of TiO_2-NPs and reproduction.

Keywords : human spermatozoa, DNA damage, TiO_2-NPs, biomarkers
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