

Shape-dependent effects of ZnO nanoparticles on redox status, transcriptomic profile, and histopathology in chicken embryos

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In recent years, the use of nanomaterials has increased markedly. To date, nanoparticles (NPs) have been widely applied as food additives, components of food packaging, and ingredients in cosmetic products. Zinc oxide nanoparticles (ZnO NPs) are among the nanomaterials most frequently used in commercial products. Due to their hydrophilic nature and solubility in biological media, they have often been regarded as safe. Nevertheless, earlier studies have shown that these nanoparticles can penetrate biological barriers such as the blood–brain barrier and, importantly, the placental barrier, thereby potentially influencing embryonic development [1].

In the present study, the effects of ZnO NPs on embryonic development were evaluated. In a chicken embryo model, the effects of nanoparticle dose and shape (oval vs. long) on developmental processes were investigated. The nanoparticles were administered as suspensions into the fertilised egg to air chamber on the first day of incubation at concentrations of 10 and 100 µg/ml. Their possible influence on embryonic redox balance was assessed by determining superoxide dismutase activity, the level of lipid peroxidation, and carbonylated protein content. Additionally, transcriptomic analysis was performed using microarrays. To determine whether exposure to the tested nanoparticles influenced programmed cell death in developing embryos, immunohistochemical staining for Bax, Bcl-2, and the active form of caspase-3 (CPP32) was carried out. Histopathological analysis was also performed.

Both shapes of ZnO NPs induced alterations in the redox status; however, long ZnO NPs exerted a weaker and more delayed effect on the evaluated parameters. Exposure to both nanoparticle shapes also affected the expression of numerous genes during early embryonic development, with 1487 genes differentially expressed in response to oval NPs and 548 in response to long NPs. Unexpectedly, oval ZnO NPs decreased the pro-apoptotic potential in chicken embryos. By contrast, long ZnO NPs did not induce changes in the presence or localisation of Bax, Bcl-2, or CPP32. Histopathological examination showed no detectable effect of the analysed nanoparticles on embryonic tissue development, regardless of nanoparticle shape or dose.

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[1] Kielbik et al. (2017) *Nanomedicine* 13, 843–852.

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