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Ultrastructure of mouse granulosa cells exposed in vitro to the fungicide Mancozeb

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Mancozeb is an ethylene bis-dithiocarbamate widely used as fungicide, also due to a low reported toxicity in mammals. However, reproductive toxicity has been demonstrated in vivo and in vitro in mouse oocytes, by the alteration of spindle morphology [1] and impairment of fertilizability [2]. Mancozeb exerted on mouse GCs cultured in vitro a premalignant-like status, indicated by reduced p53 expression [3] and a mild oxidative stress [4]. However, presence and extent of ultrastructural alterations induced in vitro by Mancozeb on GCs were not yet studied. To this aim, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) were applied on mouse GCs cultured with increasing concentration of Mancozeb. GCs were obtained by puncturing antral follicles of PMSG-treated prepubertal CD1 female mice and cultured in vitro in DMEM+5%FBS+pen/strep without (control) or with increasing concentration of Mancozeb (0.001-to-1 µg/ml) for 48hrs, at 37°C and 5%CO₂. At the end of the culture period, cells were washed in PBS, fixed in 2.5% glutaraldehyde/PBS and stored at 4°C until processing. GCs were, then, subjected to standard preparative for TEM [5] and SEM [6] observation. Results showed a dose-dependent toxicity of Mancozeb on mouse GCs. Ultrastructural data showed intercellular communication retraction, irregular nuclear membrane and chromatin marginalization at lower concentrations; chromatin condensation, membrane blebbing and cytoplasmic vacuolization at higher concentrations. In conclusion, Mancozeb showed a dose-dependent harmful effect on granulosa cells in vitro, probably due to the toxic breakdown product ethylenethiourea. TEM and SEM were again confirmed to be a valuable tool to study ultrastructural alterations after toxicants exposure.

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Keywords

Mancozeb; granulosa cells; TEM; SEM; ultrastructure.

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