TIME AND DOSE-DEPENDENT EFFECTS OF OXIDATIVE STRESS INDUCED BY HYDROGEN PEROXIDE IN OVARIAN GRANULOSA CELLS

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When the production of prooxidant molecules such as reactive oxygen species (ROS) overwhelmingly exceeding the cellular antioxidant capability, it potentially leads to a condition known as oxidative stress. Physiological level of ROS plays important roles in the reproductive process including follicular development, granulosa cell expansion, oocyte maturation, ovulation, and pregnancy. However, consistent production of an elevated level of ROS may disrupt the cellular homeostasis and results in oxidative stress. Many chemical compounds have been shown to be a potential inducer of oxidative stress in mammalian cells by increasing ROS level including hydrogen peroxide H2O2. Micromole of H2O2 is able to generate excessive ROS that may cause peroxidative damage to the cellular macromolecule and results apoptosis. The oxidative effects of H2O2 have been investigated in different types of cells, however, time and dose-dependent effects of oxidative stress induced by H2O2 in granulosa cells (GCs) have not been investigated yet. GCs were exposed to different concentrations of H2O2 (0, 100, 200, and 500 µM) for different time points (4 h, 12 h, and 24 h) and viability, cytotoxicity, and ROS accumulation was measured using trypan blue, WST-1 kit, and H2DCFDA, respectively. The results showed that there were time and dose-dependent loss of cell viability in GCs due to H2O2 exposure. For instance, lower cell viability was observed at 500 µM concentration and 24 h time point while highest viability was observed at 100 µM concentration. Similar results were observed in cytotoxicity experiment. Analysis of ROS accumulation showed a concentration-dependent increase, i.e. higher ROS at higher concentration, and time-dependent decrease, i.e. lower ROS at higher time points, of ROS accumulation in GCs. Our results confirm that higher concentration fo H2O2 is highly cytotoxic and cause a loss of cell viability of GCs by generating excessive ROS in a time and dose-dependent manner.

Keywords: Oxidative Stress, Cell Viability, Granulosa Cells