O-7(임상) Confocal Microscopic analysis of the Spindle and Chromosome Alignment of Oxidative Stress Induced Mouse Oocytes

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Background & Objectives: To evaluate the adverse effects of exogenously induced reactive oxygen species (ROS) on mouse oocyte spindle and chromosome alignment by using hydrogen peroxide as a source of ROS, and to examine the protective antioxidant supplementation (vitamin C).

Method: Mature metaphase II mouse oocytes (frozen) were exposed to various concentrations of hydrogen peroxide (H_2O_2): 12.5, 25, 50, or 200 μM. Another set of oocytes was exposed to 25 μM H_2O_2 for varying incubation times (15, 30, 45 and 60 min). Various concentration of vitamin C supplemented to culture media; 0, 50, 100, 200 and 400 μM. Immunohistochemical staining was used to evaluate the effect on oocyte microtubule morphology and chromosomal alignment. Fixed oocytes were incubated with anti-α-tubulin monoclonal antibody for microtubule staining, followed by incubation with FITC labeled anti-mouse IgG antibody. For chromosome staining, oocytes were incubated with propidium iodide. Stained oocytes were scored for alterations in microtubule morphology and chromosomal alignment under a Fluorescent (Leica, Germany) and scanning Confocal microscope (Leica Lasertechnik GmbH, Heidelberg, Germany). Scores of 1~2 were considered as being normal for oocyte microtubule morphology and chromosomal alignment, and 3~4 as abnormal (modified from Saunders and Parks, 1999).

Results: Compared to control H_2O_2 concentration significantly affected both spindle morphology and chromosome alignment. Significantly higher scores were seen for both spindle and chromosome alignment indicative of oxidative stress induced damage with > than 25 mM H_2O_2. This increase in the damaging effect was dose dependent. In addition the affect 25 mM H_2O_2 on alterations in both spindle and chromosome alignment was significantly increased with increasing period of incubation. Significant damage in spindle and chromosome alignment was observed in first 15 mins of exposure to oxidative stress. Vitamin C alone at concentrations of 400 μM did not result in damage of spindle or chromosome alignment and was similar to control. High concentrations of vitamin C was able to reduce or recovery of spindle or chromosome alignment damage caused by H_2O_2.

Conclusions: Oxidative stress lead to disruption of the MII spindle in mouse oocyte in dose and time dependent manner. This is important while handling oocytes in vitro. Reducing the exposure time during assisted reproductive techniques and antioxidant supplementation may help minimize oxidative stress and improve the quality of the oocytes.