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Can vitamin C supplementation reduce oxidative stress induced cytoskeleton damage of mouse oocyte?

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Objective: Oocytes appear to be protected against oxidative stress (OS) by oxygen scavengers that are present *in vivo*. However, when oocytes are removed from their natural environments for assisted reproduction techniques, this natural defense mechanism is lost. Earlier we demonstrated the damaging effect of oxidative stress induced by exogenous exposure to hydrogen peroxide on metaphase II oocyte microtubule and chromosomal alignment. Therefore, protecting oocytes from oxidative stress *in vitro* is important. The objective of our study was to examine the protective effect of antioxidant supplementation with vitamin C in reducing OS-induced cytoskeleton damages. **Design:** Prospective, experimental animal study
Materials and Methods: Frozen metaphase II mouse oocytes (Embryotech Laboratories Inc., Wilmington, MA) were divided into 4 groups: group I = Control with human tubal fluid only; group II = 25 μM of H_2O_2 ; group III = 100 μM vitamin C; group IV = vitamin C + H_2O_2 . For microtubule staining, fixed oocytes were incubated with anti- α -tubulin monoclonal Ab followed by incubation in FITC labeled anti-mouse IgG Ab. 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 normal and 3-4 as abnormal for microtubule morphology and chromosomal alignment (modified from Saunders and Parks, 1999). **Results:** Vitamin C alone at concentrations of 100 μM did not damage the microtubule morphology or chromosome alignment which were similar to control. Compared with control group, 25 μM of H_2O_2 exposure resulted in a significant damage to both microtubule morphology and chromosomal alignment ($P < 0.001$; Fig. 1). Vitamin C failed to reduce the damage caused by H_2O_2 .

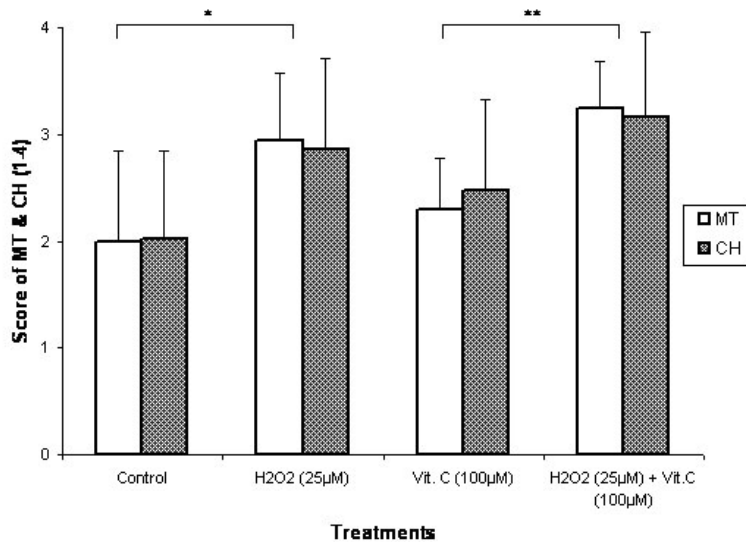


Fig. 1. Effect of vitamin C alone and in combination with H₂O₂ on alterations in microtubule structure and chromosomal alignment of mouse metaphase II oocyte; *P significantly different (<0.001) between control and 25 µM of H₂O₂, **significantly different (<0.001) between 100 µM vitamin C and 100 µM vitamin C + 25 µM of H₂O₂. Conclusion: Hydrogen peroxide presence during *in vitro* culture media of mouse oocytes can damage the oocyte cytoskeleton. Vitamin C at 100 µM is not effective in reducing the damage. Cytoskeleton damage from oxidative stress may be irreversible and/or higher concentration and longer incubation of antioxidants (such as Vitamin C) may be necessary to reduce the damage. Support: None

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