Oxidative stress-induced alterations in Metaphase-II mouse oocyte spindle structure and beneficial effects of vitamin C supplementation in vitro

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Oxidative stress can alter oocyte quality and lead to reduced fertility outcomes in vitro. Lack of antioxidant defense in the assisted reproduction media may be one of the causes of increased oxidative insult on the oocytes. Damage induced by oxidative stress on the spindle structure that consists of microtubules and chromosomes may not be apparent in morphologically normal oocytes under light microscopy. This may lead to selection of poor quality oocyte for assisted reproduction techniques. The objective of this study was: 1) to examine the concentration and exposure time effects of exogenously induced oxidative stress using hydrogen peroxide (H$_2$O$_2$) on the microtubule structure and chromosomal alignment in metaphase-II (M-II) mouse oocytes and 2) study the effect of supplementing the media with various concentrations of vitamin C for its beneficial effects in conferring protection or reducing the oxidative stress induced damage.

Mature M-II mouse oocytes were exposed to various concentrations of H$_2$O$_2$: 12.5, 25, 50, and 200 µM and another set exposed to 25 µM H$_2$O$_2$ for varying incubation times (15, 30, 45 and 60 min). Vitamin C concentrations of 0, 50, 100, 200 and 400 µM were supplemented to culture media with 25 µM H$_2$O$_2$. Controls consisted of incubation with culture media alone. 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, propidium iodide for chromosome staining. Alterations in microtubule morphology and chromosomal alignment were scored using epifluorescence and scanning confocal microscopy. Scores of 1-2 were considered as normal for both microtubule morphology and chromosomal alignment, and 3-4 as abnormal.

Compared to controls, significantly higher scores indicative of oxidative stress induced damage were seen for both microtubule and chromosome alignment with > 25 µM H$_2$O$_2$ (p < 0.0001). This increase in the damaging effect was both dose and time dependent. Vitamin C alone at all concentrations did not damage microtubule morphology or chromosome alignment and effects were similar to controls. Preincubation with vitamin C before inducing exposure to H$_2$O$_2$ did not appear to confer any protective effect, however simultaneous co incubation of vitamin C with H$_2$O$_2$ appeared to significantly reduce the toxic effect of H$_2$O$_2$.

Oxidative stress leads to disruption of the M-II 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 may be beneficial for obtaining better quality oocyte. The supplementation of vitamin C in the media to protect or reverse the effects of oxidatively damaged oocyte microtubular morphology and chromosomal alignment requires further study.