Objective: Oxidative stress as a result of increased formation of reactive oxygen species or reduced scavenging ability of antioxidants in vitro culture can compromise oocyte spindle quality and affect the outcome of assisted reproduction. Exogenous exposure to hydrogen peroxide (25 µM) for 30 min has been shown by us to significantly induce spindle structure alterations. The objective of this study was to examine the protective effect of coincubating metaphase-II mouse oocytes with vitamin C or pentoxifylline in protecting spindle structure (microtubule morphology and chromosomal alignment) from exogenous effects of oxidative stress.

Design: Prospective study

Materials and Methods: Cryopreserved mouse oocytes were thawed and transferred into phosphate buffered saline (pH 7.4) for 10 minutes at room temperature to remove excess cryoprotectant and for equilibration. Metaphase-II oocytes were incubated in HTF (human tubular fluid) at 37°C with 5% CO2 for an hour allowing the spindles to repolymerize. The oocytes (6-8 per dish) were grouped as i) control: with HTF only; ii) pentoxifylline (500 and 1000 µM); iii) vitamin C (100, 200, and 400 µM); iv) H2O2 (25 µM); v) H2O2 + vitamin C (100, 200, 400 µM) and vi) H2O2 + pentoxifylline (500, 1000 µM).

Fixed oocytes were incubated with anti-α-tubulin monoclonal antibody followed by incubation with FITC labeled anti-mouse IgG antibody for staining microtubules and propidium iodide for chromosomes. Microtubule morphology and chromosome alignment was scored using epifluorescent microscope. A score of 1-2 was considered normal (good outcome), whereas 3-4 abnormal (poor outcome).

Results: Hydrogen peroxide at 25 µM demonstrated significant alterations in both microtubule structure and chromosomal alignment. Both vitamin C and pentoxifylline alone did not affect the microtubule or chromosome alignment at any of the concentrations examined and was not statistically different from the control group. Oocyte coincubated with hydrogen peroxide + 200 µM vitamin C significantly reduced the microtubule and chromosomal damage (scores <3). Coincubation of oocytes with H2O2 + pentoxifylline (500 µM) appeared to show slight improvement in chromosomal alignment (P<0.08) only.

Conclusion: Use of antioxidant such as vitamin C (200 µM) during in vitro handling of oocytes may confer protection against oxidative insult to spindle structure. Potential benefits of pentoxifylline are not very promising and the concentrations that may be effective need to be carefully examined. Methods aimed at reducing oxidative stress in external cultures may be included in protocols to optimize oocyte quality in assisted reproduction.
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