Oxidative stress induced alterations in the mouse oocyte cytoskeleton

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Objective: Oxidative stress may induce alterations in the cytoskeleton of the oocytes. Damage to the cytoskeleton in mature oocyte, especially the spindle, which is comprised of microtubules, can result in failure of the final meiotic reduction division. We examined the alterations in the cytoskeleton and chromosome alignment of the metaphase II (MII) oocytes under conditions of oxidative stress induced in vitro. Design: Prospective, experimental animal study Materials and Methods: Mature metaphase II mouse oocytes (frozen) were exposed to various concentrations of hydrogen peroxide (H₂O₂): 12.5, 25, 50, or 200 µM. Another set of oocytes was exposed to 25 µM H₂O₂ for varying incubation times (15, 30, 45 and 60 min). 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 controls (Fig. 1A) H₂O₂ concentration significantly affected both spindle morphology and chromosomal alignment. Significantly higher scores were seen for both microtubule and chromosome alignment indicative of oxidative stress induced damage with >25 µM H₂O₂. This increase in the damaging effect was dose dependent. In addition the effect of 25 µM H₂O₂ on alterations in both microtubule and chromosome alignment was significantly increased with increasing period of incubation. Significant damage in microtubule and chromosome alignment was observed in first 15 mins of exposure to oxidative stress.
Fig. 1. Confocal microscopic photomicrographs of microtubule and chromosome in mouse oocyte (metaphase II) (A): showing normal characteristic barrel shaped spindle structure, (B-C) damaging effect of 25 and 50 µM H₂O₂ exposure for 30 min showing alterations in microtubule and chromosome alignment, and (D-F): changes following 25 µM H₂O₂ exposure for 30, 45 and 60 min.

Conclusion: Oxidative stress leads 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 may help minimize oxidative stress and improve the quality of the oocytes. Support: None

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