Effect of Tumor necrosis factor induced alterations in microtubule and chromosomal alignment of metaphase II oocyte - possible role in endometriosis associated infertility

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Objective: Endometriosis, even in a mild stage, may have a direct negative effect on oocyte development, fertilization and embryogenesis. Tumor necrosis factor (TNF)-α is a pleiotropic cytokine with immune-regulating properties. It appears to act as the switch point in the cascade of immunologic processes during endometriosis. Poor quality of oocyte from endometriosis patient may be affected by the presence of TNF-α in the peritoneal fluid. TNF-α receptors are reported to be present on the oocyte. The objective of our study was to examine the dose-dependent effect on microtubule morphology and chromosomal alignment in cryopreserved metaphase II oocytes.

Design: Prospective, experimental animal study Materials and Methods: Mature metaphase II oocytes were divided into 5 groups and exposed to mouse TNF-α concentrations prepare in human tubal fluid (HTF): 100, 200, 400 and 600 ng/mL. Controls consisted of an equal volume of HTF. For microtubule staining, fixed oocytes were incubated in anti-α-tubulin monoclonal Ab followed by incubation in FITC labeled anti-mouse IgG Ab. For chromosome staining, oocytes were incubated in 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: TNF-α resulted in alterations in both microtubule morphology and chromosomal alignment. The effect was visible at 200 ng/mL concentration of TNF-α (Fig. 1).
Fig. 1. Affect of TNF-α on microtubule and chromosomal alignment of mouse metaphase II oocytes
Some of affected oocytes displayed microtubules with typical helmet-like appearance and the characteristic barrel shape was lost (Fig. 2A). Chromosomal alignment was significantly disorganized with higher concentration of TNF-α (Fig. 2B-C).

Fig. 2. Representative confocal microscopic photomicrographs of (A): normal spindle showing characteristic microtubule and chromosome alignment in mouse oocyte (metaphase II) and (B): alterations following 400 ng/mL TNF-α and (C): 600 ng/mL TNF-α. Conclusion: TNF-α can damage spindle structure and cause alterations in microtubule and chromosomal alignment in mouse oocyte. The varying response of oocytes to TNF-α may be due to the presence of different amounts of TNF-α receptors. This may be one of the many causes of poor oocyte quality obtained from endometriosis patients. Use of anti-TNF-α drugs may be beneficial in reducing/reversing these changes. Support: None
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