

**Conclusions:** Our data indicate a possible role of EA in the regulation of the female endocrine function and therapeutic approach to overcoming anovulation in women with PCOS.

## **P-9      The Effect of Tumor Necrosis Factor on the Microtubule and Chromosomal Alignment in the Mouse Oocytes**

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**Background & Objectives:** Endometriosis, even in a mild stage, may have a direct negative effect on oocyte development, fertilization and embryogenesis. Tumor necrosis factor 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 tumor necrosis receptors are reported to be present on factor in the peritoneal fluid. TNF- $\alpha$  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.

**Method:** Mature metaphase II oocytes were divided into 5 groups and exposed to mouse TNF- $\alpha$  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 are incubated in anti-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).

**Results:** Tumor necrosis factor resulted in alterations in both microtubule morphology and chromosomal alignment. The effect was visible at 200 ng/mL concentration of tumor necrosis factor. Some of affected oocytes displayed microtubules with typical helmet-like appearance and the characteristic barrel shape was lost. Chromosomal alignment was significantly disorganized with higher concentration of tumor necrosis factor.

**Conclusions:** Tumor necrosis factor can damage spindle structure and cause alterations in microtubule and chromosomal alignment in mouse oocyte. The varying response to tumor necrosis factor may be due to the presence of different amounts of tumor necrosis factor receptors. This may be one of the many causes of poor oocyte quality obtained from endometriosis patients. Use of anti-tumor necrosis drugs may be beneficial in reducing/reversing these changes.