Objective: Normal spindle structure consisting of microtubule and chromosomes is associated with successful cell division and subsequently embryo development. Numerous studies have identified tumor necrosis factor (TNF-α) and reactive oxygen species to be increased in peritoneal fluid of women with endometriosis. The objective of the study was to examine the individual and combined effect of exogenous exposure to hydrogen peroxide (H₂O₂) and TNF-α during the in-vitro culture system on the oocyte spindle structure. Design: Prospective in vitro study Materials and Methods: Previous studies from our group have shown that 25 µM concentrations of H₂O₂ and 200 ng/mL TNF-α affect the oocyte spindle structure. Mouse metaphase II oocytes (Embryotech Laboratories Inc. Wilmington, MA) were incubated after thawing at 37°C for 30 mins. Oocytes were divided into 4 groups: group I: Control incubated with human tubal fluid (HTF) alone, group II: 25 µM H₂O₂, group III: 200 ng/mL TNF-α, and group IV: H₂O₂ (25 µM) + TNF-α (200 ng/mL). Following incubation for 30 min in 37°C, immunohistochemical staining was done to evaluate the effect on oocyte microtubule and chromosome alignment. Fixed oocytes were incubated with anti-α-tubulin monoclonal antibody followed by incubation with FITC labeled anti-mouse IgG antibody. For chromosome staining, oocytes were incubated with propidium iodide. Microtubule morphology and chromosome alignment was scored and a score of 1-2 was considered normal, whereas 3-4 abnormal (modified from Saunders and Parks, 1999). Results: Higher scores for changes in microtubule morphology and alteration in chromosome alignment were seen following exposure to H₂O₂, TNF-α and H₂O₂ + TNF-α compared with control (P < 0.05, Fig. 1). Significantly higher incidence of abnormal microtubule morphology was seen in H₂O₂ alone group compared with TNF-α alone group (3.16 ± 0.78 vs. 2.71 ± 0.52, P = 0.009). Also, compared with both TNF-α alone group, and H₂O₂ + TNF-α group, a significantly higher score was seen for microtubule morphology in H₂O₂ + TNF-α group (3.19 ± 0.63 vs. 2.71 ± 0.52, P = 0.003, Fig. 1). Changes in chromosomal alignment were comparable between the 3 groups.
Figure 1. Effect of exogenous exposure to H$_2$O$_2$ and TNF-α alone and in combination on alterations in microtubule (MT) structure and chromosomal alignment (CH).

Conclusion: Oxidative stress as a result of exposure to hydrogen peroxide or TNF-α results in alterations in microtubule and chromosomal alignment. Compared to TNF-α, hydrogen peroxide alone or in combination with TNF-α has more deleterious effects on the microtubule structure. This may explain the poor oocyte quality seen in many women with endometriosis where elevated TNF-α levels may be seen in response to oxidative stress. Support: None

Author Disclosure Block:  W. Choi, None; J. Banerjee, None; A. Agarwal, None; W. Paik, None; T. Falcone, None; R.K. Sharma, None.

Category (Complete): Endometriosis (GPC)
Keyword (Complete): endometriosis ; oxidative stress ; tumor necrosis factor-alpha (TNF-a) ; oocyte
Additional (Complete):
    Presenting Author Fellow : No
    In-Training Award : True
    ACCME Disclosure : I will not be discussing non-FDA approved products
    I Agree : True

Status: Complete