

## O-13                      Trophoblast Apoptosis Is Increased In Women With Evidence of Th-1 Immunity

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**Background & Objectives:** Accumulating evidence suggests that Th-1 immunity to trophoblast may be involved in some cases of recurrent spontaneous abortion (RSA). Previous studies have shown that placental apoptosis was increased in tissues obtained from women with RSA compared to normal controls, but cause versus effect phenomena for this finding has not been defined. In the present study, we hypothesized that women with evidence of Th-1 immunity to trophoblast would be more likely to induce apoptosis in the trophoblast cell line, JEG-3 cells.

**Method:** Two types of experiments were designed: 1) detection of Th-1 cytokine, IFN- $\gamma$  in the supernatants of peripheral blood mononuclear cells (PBMCs) from women with unexplained RSA and fertile controls stimulated with an antigen extract derived from JEG-3 cells as previously described and 2) detection of apoptosis in trophoblast cells (JEG-3 cells) treated with the supernatants. PBMCs were isolated from 380 nonpregnant women with unexplained RSA and 10 nonpregnant fertile controls and cultured for four days in the presence of an antigen extract from the trophoblast cell line, JEG-3 cells. The levels of IFN- $\gamma$  were detected by ELISA. Samples from women with RSA were divided into two groups depending upon whether IFN- $\gamma$  was increased more than 2-fold over PBMC culture supernatants from unstimulated culture supernatants (IFN- $\gamma$  positive group, IFN- $\gamma$  negative group). JEG-3 cells were cultured in the presence of supernatants from 40 patients of IFN- $\gamma$  positive group, 40 patients of IFN- $\gamma$  negative group and 10 persons of fertile controls and then apoptosis was detected using the Apoptag kit (Intergen). Student's t-test was used to determine statistical significance.

**Results:** The level of IFN- $\gamma$  secreted in the supernatants of PBMCs from women with unexplained RSA stimulated with an antigen extract was significantly higher than that from the normal control group. Among the RSA patients, the incidence of apoptosis of trophoblast cells in IFN- $\gamma$  positive group was higher than in IFN- $\gamma$  negative group as well as the normal control group ( $p < 0.05$ ).

**Conclusions:** Apoptosis was significantly increased in JEG-3 cells cultured in the presence of supernatants from women with evidence of Th-1 immunity to trophoblast suggesting a possible pathological mechanism of Th-1 immunity associated reproductive failure.