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**Topic :** Endometriosis and the peritoneum

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An original in vitro model of endometrial cell adhesion to peritoneum: the early stage of pelvic endometriosis development

Endometriosis is defined as the presence of endometrial tissue outside the uterine cavity. Sampson's theory states that menstrual endometrial cells pass retrogradely through the fallopian tubes, attach and grow on peritoneal surfaces, leading to pelvic endometriotic lesion development.

In this study, we set up an original in vitro co-culture model, allowing quantification of human endometrial cell attachment to human peritoneal mesothelial cells obtained from the same patients. This original model may help us to better understand the adhesion process in endometriosis and to assess the effect of several molecules.

Endometrial and peritoneal samples were collected from patients undergoing laparoscopy for benign conditions. Endometrial and mesothelial cells were both enzymatically isolated using collagenase IA and cultured. At confluence, endometrial cells were collected and labeled with a fluorescent tracker (CFDA-SE). Labeled endometrial cells were then plated over confluent monolayers of mesothelial cells in 96-well plates at 5,000 to 40,000 cells per well. After 1 hour of co-culture, non-adherent endometrial cells were eliminated by washing, and fluorescence was measured to assess endometrial cell attachment to peritoneal mesothelial cells.

Culture purity was immunohistochemically evaluated using monoclonal antibodies to human CD10 and CK39, which are specific markers for endometrial and mesothelial cells respectively. The isolation procedure ensured cell purity of at least 90% for both cell types.

Endometrial cell labeling was also validated by fluorimetry, which showed a linear correlation with the number of labeled endometrial cells over a range of 500 to 50,000 cells ( $r^2=0.99$ ).

Adhesion assays showed fluorescence readings that were linearly proportional to the number of endometrial cells placed on mesothelial cells per well over a range of 5,000 to 40,000 cells ( $r^2=0.93$ ).

The pathogenesis of early endometriotic lesion formation can be divided into three crucial events: attachment of endometrial cells to peritoneal mesothelial cells, transmesothelial invasion, and proliferation in the submesothelial extracellular matrix.

We developed an original in vitro model of endometrial cell attachment to confluent mesothelial cells obtained from the same patients. This quantitative model facilitates study of the adhesion process in endometriosis and will allow us to compare attachment of endometrial cells from women with and without endometriosis, and to evaluate the role of hormones, cytokines, and growth factors.