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Cytokine Profiling in Cultured Endometrial Cells after Hormonal Treatment

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Abstract : The human endometrium-myometrium interface (EMI) is the uterine inner barrier without a separatig layer. It is composed of endometrial epithelial cells (EEC) and endometrial stromal cells (ESC) in the endometrium and myometrial smooth muscle cells (MSMC) in the myometrium. The EMI undergoes structural remodeling during the menstruation cycle which are essential for human reproduction. Recently, we co-cultured a layer-by-layer in vitro model of EEC, ESC and MSMC on a synthetic membrane for mechanobiology experiments. We also treated the model with progesterone and β-estradiol in order to mimic the in vivo receptive uterus In the present study we analyzed the cytokines profile in a single layer of EEC the hormonal treated in vitro model of the EMI. The methodologies of this research include simple tissue-engineering . First, we cultured commercial EEC (RL95-2, ATCC® CRL-1671™) in 24-wellplate. Then, we applied an hormonal stimuli protocol with 17-β-estradiol and progesterone in time dependent concentration according to the human physiology that mimics the menstrual cycle. We collected cell supernatant samples of control, pre-ovulation, ovulation and post-ovulaton periods for analysis of the secreted proteins and cytokines. The cytokine profiling was performed using the Proteome Profiler Human XL Cytokine Array Kit (R&D Systems, Inc., USA) that can detect105 human soluble cytokines. The relative quantification of all the cytokines will be analyzed using xMAP – LUMINEX. We conducted a fishing expedition with the 4 membranes Proteome Profiler. We processed the images, quantified the spots intensity and normalized these values by the negative control and reference spots at the membrane. Analyses of the relative quantities that reflected change higher than 5% of the control points of the kit revealed the The results clearly showed that there are significant changes in the cytokine level for inflammation and angiogenesis pathways. Analysis of tissue-engineered models of the uterine wall will enable deeper investigation of molecular and biomechanical aspects of early reproductive stages (e.g. the window of implantation) or developments of pathologies.

Keywords : tissue-engineering, hormonal stimuli, reproduction, multi-layer uterine model, progesterone, β-estradiol, receptive uterine model, fertility
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