T-039 - Endometrial Stromal Cells Acutely Exposed to DEHP Do Not Recapitulate Alterations Observed in Endometriosis.

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**Abstract**

**Introduction:** Acute exposure to Di(2-ethylhexyl)phthalate (DEHP) in primary endometrial stromal cells (ESC) is used to model endometriosis because it replicates some endometriosis phenotypes (increased cell viability, invasiveness, immune response, and altered steroid receptor expression) [Sung-Hoon et al. (2015), Cho et al. (2015)]. We sought to further characterize the *in vitro* effects of an acute DEHP exposure in ESC, focusing on potential epigenetic deregulation. We hypothesized that DEHP exposure could induce changes in ESC that replicate all the phenotypic alterations described in endometriosis.

**Methods:** Primary ESC were isolated by gravity sedimentation from endometrial biopsies collected from healthy oocyte donors, the day of ovarian puncture (n=4). ESC were exposed to DEHP (1-25 µM) or vehicle (0.002% DMSO) for 0, 24, 48, and 72 hours (h). Cell viability was measured at each time point by cell proliferation assay (MTS, Promega). mRNA expression was measured by qPCR (n=3; StepOnePlus Real-Time PCR System, Applied Biosystems) for markers of inflammation (*IL6*), angiogenesis (*VEGFA*), endometrial morphogenesis (*HOXA10*), and epigenetic modulation (*KDM1A* and *EZH2*), all previously described as altered in the eutopic endometrium of women with endometriosis, in ESC exposed to DEHP (1-25 µM) or vehicle for 48 h. Invasiveness was measured in ESC exposed to DEHP (10 µM) or vehicle with FBS 0% for 24h. Then, the cells were re-seeded on the cell invasion assay (Cell Biolabs, Inc) inserts, with FBS 10% as invasion stimulus over 24 h.

**Results:** We did not find any statistically significant difference in ESC cell viability (n=4) or gene expression (n=3) post-acute exposure to DEHP (1-25 µM). *HOXA10* gene expression was significantly decreased, similar to that described in endometriosis, at DEHP 25 µM (p = 0.0174). ESC invasiveness (n=4) post-acute exposure to DEHP (10 µM) exhibited a non-significant increase.

**Conclusion:** Our observations suggest that acute exposure (0-48 h) of ESC to DEHP (1-25 µM) does not trigger all the phenotypic alterations described in the eutopic endometrium of women with endometriosis. Therefore, we do not consider it a good model to study the *in vitro* alterations present in endometriosis. Funded by a grant from the IVI Foundation, Miguel Servet Contract (CP013/0450) and ISCIII FIS project (PI17/00931).