Exposure to Phthalates Disrupts Ovulatory Luteinizing Hormone/Choriogonadotropin Receptor Signaling and Decreases Downstream Prostaglandin Production in Human Granulosa Cells *In Vitro*

Katie Land[1,2]; Hong Xu[2]; James Akin[3]; Patrick Hannon[1,2]

1. Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY, United States
2. Department of Obstetrics and Gynecology, University of Kentucky, Lexington, KY, United States
3. Bluegrass Fertility Center, Lexington, KY, United States

Humans are ubiquitously exposed to phthalates, which are widely used chemicals that disrupt female reproductive health. Phthalates are a family of solvents and plasticizers found in commonly used products including cosmetics, food/beverage containers, medical tubing, and car upholstery, among other sources. Phthalates undergo rapid metabolism in the body, and these metabolites can target the ovary. Due to their classification as endocrine-disrupting chemicals, exposure to phthalates and their active metabolites could impair ovulation. To best mimic human exposure, this study used an environmentally relevant phthalate metabolite mixture (MPTmix), which was derived from urinary phthalate measurements in pregnant women. The ovulatory process is initiated by an endogenous surge of luteinizing hormone (LH) or clinical treatment with human chorionic gonadotropin (hCG, a potent LH analogue). LH/hCG binding to its receptor (LHCGR) on ovarian granulosa cells promptly activates various intracellular signaling cascades, including pathways mediated by protein kinase A (PKA), protein kinase B (AKT), and extracellular signal-regulated kinase 1/2 (ERK1/2). Additionally, it is postulated that ERK1/2 signaling drives the production of prostaglandin E_2 (PGE_2) via transcription of *PTGS2*, a prostaglandin synthase. Increases in PGE_2 are vital for the inflammatory changes that occur during ovulation, including cumulus-oocyte complex expansion, follicle rupture, and angiogenesis. We hypothesized that MPTmix exposure would disrupt essential signaling cascades involved in ovulation (PKA, AKT, ERK1/2), alter mRNA levels of *PTGS2* and enzymatic activity of PTGS2 protein, and decrease PGE_2 levels. Granulosa-lutein cells from follicular aspirates of women undergoing *in vitro* fertilization were acclimated in culture to regain LH/hCG responsiveness. Prior to hCG treatment, cells were exposed for 48hr to vehicle control (dimethylsulfoxide, DMSO) or varying doses of the MPTmix (1-500µg/ml). Following treatment ± hCG and ± MPTmix, cells and media were collected at 0, 0.5, 6, 12, 24, or 36hr to measure the levels of cAMP (upstream of PKA), p-PKA, p-AKT, p-

ERK1/2, *PTGS2* mRNA, PTGS2 activity, and PGE_2 levels (n=3-11; p≤0.05). Treatment with hCG alone rapidly (0.5hr post-treatment) increased cAMP, p-PKA, p-AKT, and p-ERK1/2 levels when compared to DMSO. However, treatment with hCG+MPTmix 500µg/ml decreased cAMP, p-PKA, and p-ERK1/2 levels relative to the hCG ovulatory control group at 0.5hr. Similarly, hCG treatment increased *PTGS2* mRNA levels and PTGS2 activity at all timepoints when compared to DMSO alone. Treatment with hCG+MPTmix decreased *PTGS2* mRNA levels at 6hr (500µg/ml), 12hr (1, 10, 500µg/ml), 24hr (all doses), and 36hr (100, 500µg/ml) when compared to hCG. Additionally, hCG+MPTmix treatment decreased PTGS2 activity at 24hr (all doses) and 36hr (1, 100, 500µg/ml) relative to hCG. The decreases in PTGS2 activity correlate with the 24hr and 36hr MPTmix-induced decreases (all doses) in PGE_2 levels compared to hCG. These data demonstrate that phthalate exposure alters requisite ovulatory signaling cascades, and has further downstream inhibitory effects on mediators that drive functional changes leading to oocyte release. These findings in human granulosa cells exposed to an environmentally relevant mixture of phthalates suggest that phthalates may impair the ovulatory process in women. Supported by R01ES033767, R00ES028748, P30ES026529.