Molecular Mechanisms Underlying the Adverse Outcomes of DEHP Exposure on Human Preovulatory Follicles

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Di(2-ethylhexyl) phthalate (DEHP) is a well-known plasticizer and an endocrine disrupter that enters the human body due to leaching from plastic objects from everyday use. It is extensively metabolised upon uptake forming a range of hydrolysed and oxidized metabolites that are considered as the active, endocrine disruptive forms. While DEHP metabolites have been long investigated in relation to male infertility, several rodent studies have raised a concern that DEHP also affects ovarian folliculogenesis and steroid hormone production, thereby affecting fertility in females. Our previous study found a significant association between DEHP metabolite levels in the human follicular fluid (FF) and reduced responsiveness to controlled ovarian stimulation during IVF treatments. Here, we investigated the mechanisms underlying these effects though molecular analysis of follicular fluid samples.

To explore the molecular cues altered by DEHP exposure in humans, samples from 96 IVF patients from Swedish (N=48) and Estonian (N=48) infertility clinics were analysed. The patients were stratified into high (mean 7.7 ± SD 2.3 nM, N=48) and low (0.8 ± 0.4 nM, N=48) DEHP exposure groups according to the molar sum of the measured levels of 4 DEHP metabolites MEHP, MEHHP, MEOHP and MECPP in their FF samples. Extracellular miRNA levels and 15 steroid hormone concentrations were measured from the same samples by small RNA sequencing and LC-MS/MS, respectively. In addition, FF somatic cell samples from the individual follicles were available in the Estonian cohort, and these were used to measure mRNA expression levels by RNA-seq.

The obtained results were compared between the high and low DEHP groups, using the cohort as a covariate. In addition, data integration was used to understand the interactions between the disturbed miRNA, mRNA and steroid levels within a single follicle.

Differential expression (DE) and miRNA:mRNA network analysis revealed that the expression levels of the majority of genes in the cholesterol biosynthesis and steroidogenesis pathways were significantly decreased (FDR<0.05). The DE miRNAs (FDR<0.1) were predicted to target a considerable number of key enzymes in these pathways (FDR<0.05). The down-regulation of CYP17A1 (log_2FC=-2.34, FDR=0.022) in the somatic cells was linked to a reduced progesterone to 17-OH-progesterone ratio (p=0.046) in the FF of women in the high DEHP group. The expression levels of genes involved in inflammatory processes were significantly elevated in the women from the high DEHP group (FDR<0.05). Following a computational cell-type deconvolution approach, an indication for the

infiltration of cells expressing leukocyte markers of M1 and M2 macrophages, T-cells ($p<0.01$), as well as neutrophils ($p<0.05$) was observed.

Our results conclude that the exposure to DEHP metabolites significantly alters the follicular milieu within human ovaries: the inflammatory environment is stimulated, while lipid and steroid synthesis are obstructed. This study contributes to understanding the molecular mechanisms through which DEHP exposure may disrupt ovarian sensitivity to pituitary hormones in humans.