1507 Differential Gene Expression Analysis in Mouse Placentae Reveals Association between Preterm Birth Linked Genes and $PM_{2.5}$ Exposure

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Numerous epidemiological and animal studies have demonstrated that exposure to ambient fine particulate matter (<2.5µm in diameter [$PM_{2.5}$]) during gestation is associated with adverse obstetric outcomes including preterm birth (PTB). Early delivery has been linked to several lifetime health consequences for offspring, including behavioral and psychological abnormalities and reduced immune and respiratory functions. In a previous study performed in this laboratory, B6C3F1 pregnant mice exposed to concentrated ambient PM (CAPs) by inhalation, demonstrated shortened (by 0.4 d) gestational duration compared to filtered air (FA) controls. The mechanisms underlying the association between $PM_{2.5}$ and PTB are not currently well understood. Since the placenta provides a crucial link between the intrauterine environment and fetal growth/development, it is a major target of PM and key for studying the effects on birth outcomes. Therefore, in this study, placentae from the previously developed pregnant mouse model (n=6 each from CAPs and FA groups) were subjected to whole transcriptomic profiling by RNAseq. A bioinformatic RNAseq analysis workflow (tximport, Salmon and edgeR /limma-voom) was used to identify differentially expressed genes between treatment groups. The 648 genes from the curated dbPTB (database Preterm Birth) were used for a candidate gene approach and were examined using gene counts obtained from RNAseq. Following $PM_{2.5}$ exposure, six PTB genes were downregulated in placentae (Ace, Ddah1, Col1a2, Chst15, Akap12, Ephx1) and one was upregulated (Chys3) ($p<0.01$). Gene Ontology demonstrated that these seven genes are involved in neutrophil-mediated immunity, arterial blood pressure regulation, amino acid binding, cell membrane function, metal ion binding, and aromatic compound catabolism among other functions that could be linked to PTB. Additional computational models, agnostically testing placenta transcriptome-wide differential gene expression, revealed additional genes that were differentially expressed between the two treatment groups. These findings suggest that PM exposure influences the placenta genome thereby mediating PTB. Identification of these differentially expressed genes may contribute to intervention strategies to mitigate these adverse effects. *Supported by: March of Dimes, NIEHS P30ES000260, and NIEHS P30ES023515.*

1508 Stainless Steel Welding Fumes Adversely Affect Migratory Ability of First Trimester Human Placental Cells

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In the United States, the number of female welders has increased by more than two percent over the past decade. The U.S. Bureau of Labor Statistics predicts steady industry growth through 2024, and it is expected that many women will continue to fill these roles as the baby-boomer generation prepares to retire. Not surprisingly, very little data currently exists on the potential reproductive effects of mild and stainless steel welding fumes on the placenta. Using human placental trophoblast cells (HTR-8/SVneo) from the first trimester, we aimed to identify the mechanisms of toxicity associated with stainless steel (SS) and mild steel (MS) welding rods. MS welding fumes are mainly comprised of iron and manganese, while SS welding fumes primarily contain hexavalent chromium and nickel. During embryogenesis and placentation, cellular migration is a highly orchestrated and multi-step process that plays an integral role in providing the foundation for a successful pregnancy. In this study, exposure of HTR-8/SVneo cells to 100 µg/ml of SS welding fumes for 24 h using the Radius™ Migration assay showed significant inhibition of cellular migratory ability, whereas cells exposed to MS were not affected. Using electron paramagnetic resonance, exposure of cells to SS also produced greater amounts of the hydroxyl radical when compared to MS. Results from a multiplex cytokine kit (Meso Scale Diagnostics, LLC) show that exposure of cells to SS causes a pro-inflammatory response, with significant increases of IL-8 and IL-15 observed. Finally, scanning electron microscopy was performed to better understand how particles are internalized by placental cells. For both MS and SS, welding fumes accumulated in vascular spaces, which could potentially explain the increased Endothelin-1 results observed using ELISA. Endothelin-1 is a potent vasoconstrictor that is necessary for fetal formation, but upregulated in the setting of inflammation. Our data shows that SS appears to have the most damaging effect on placental cells, which could be due the presence of hexavalent chromium, not found in MS. Further studies are needed to delineate the toxicity of the individual metals found in welding fumes and their effects on the female reproductive system.

1509 Exposure to Concentrated Ambient Fine Particulate Matter ($PM_{2.5}$) Depletes the Ovarian Follicle Reserve in Mice Genetically Predisposed to Atherosclerosis

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Ovarian follicles progress from a primordial stage through primary, secondary, antral and finally preovulatory stages. The finite pool of primordial follicles constitutes the total ovarian follicle reserve. Exposure of female rodents to polycyclic aromatic hydrocarbons (PAHs) results in destruction of immature primordial and primary follicles. $PM_{2.5}$ is rich in PAHs, which are adsorbed onto particle surfaces; the effects of exposure to $PM_{2.5}$ on the ovarian reserve have not been investigated. Apolipoprotein (Apo) E null mice were exposed to concentrated ambient $PM_{2.5}$ or filtered air for 12 weeks, 5 days per week for 4h/day using a Versatile Aerosol Concentration Enrichment System. ApoE null mice are predisposed to develop atherosclerotic plaques; these mice were part of a study investigating the ovarian and cardiovascular effects of $PM_{2.5}$. Mice were euthanized on the day of proestrus of the estrous cycle 24h after the final exposure. One ovary per mouse was processed for follicle counts. Primordial and primary follicles were counted blind to treatment in every 4^{th} 20 µm section on a stereology system using the optical fractionator method. Secondary and antral follicles were followed through every section. Primary follicle numbers were significantly decreased by 51% ($P=0.007$, t-test), and primordial follicle numbers were nonsignificantly decreased ($P=0.13$) in $PM_{2.5}$-exposed mice. The overall small follicle count (primordial plus primary) was significantly decreased by 45% ($P=0.03$). Numbers of healthy secondary follicles were significantly decreased by 22% in $PM_{2.5}$-exposed mice. Antral follicle counts were not significantly affected. We assessed recruitment of primordial follicles into the growing pool by immunostaining for the mitosis marker Ki67. The percentages of primordial and primary follicles with granulosa cells positive for Ki67 were not significantly different in the ovaries from $PM_{2.5}$- versus filtered air-exposed mice. In summary, the irreplaceable ovarian follicle reserve was decreased by half in $PM_{2.5}$-exposed mice. Decreased ovarian reserve leads to premature ovarian failure, which increases the risk for cardiovascular disease in women. Thus our data provide a potential link between ovarian and cardiovascular effects of exposure to $PM_{2.5}$. Ongoing work is investigating the mechanism of follicle depletion. *Supported by California Air Resources Board, 16RD005.*

1510 The Trichloroethylene S-(1,2-Dichlorovinyl)-l-Cysteine Causes an Early Metabolic Shift Followed by Mitochondrial Dysfunction in a First Trimester Extravillous Trophoblast Cell Line

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Trichloroethylene (TCE), is an industrial solvent and widespread Superfund site contaminant. Despite epidemiological evidence associating exposure with adverse birth outcomes, the effects of TCE and its metabolite S-(1, 2-dichlorovinyl)-L-cysteine (DCVC) on the placenta remain undetermined. The appropriate balance and utilization of energy metabolism pathways and proper mitochondrial function are essential for placental cells. Our study investigated the effects of DCVC exposure on energy metabolism and mitochondrial function in placental cells. Human extravillous trophoblast cells, HTR-8/SVneo, were exposed to 5-20 µM DCVC for 6 or 12 hours. Targeted metabolomics were used to evaluate simultaneous DCVC-induced changes in metabolite concentrations from different energy metabolism pathways. Our experiments demonstrated an early increase in glycolysis as well as a partial upstream obstruction in the glycolytic pathway, resulting in lipid breakdown as a source of biofuel to provide intermediate glycolytic substrates that enter the pathway downstream of the obstruction. Additionally, an initial increase in TCA cycle activity was observed followed by a slight decrease in activity. Using a Seahorse XF Analyzer for monitoring concurrent oxygen consumption rate and extracellular acidification rate in real time, our experiments also demonstrated an increase in basal oxygen consumption, mitochondrial proton leak and decrease in energy coupling efficiency accompanied by sustained extracellular acidification indicative of glycolytic activity. These changes were followed by observed decreases in mitochondrial-dependent basal and maximum oxygen consumption rates and dissipation of mitochondrial membrane potential. Taken together, these results suggest that DCVC exposure causes substantial energy stress, necessitating alterations in biofuel sources and energy metabolism pathway utilization, further complicated by progressive mitochondrial dysfunction and resulting in decreased physiological adaptability. Our findings demonstrate the biological plausibility of DCVC-induced placental toxicity and provide new insights into the toxicological mechanisms of action of TCE and its metabolite DCVC.

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