Flavored E-Cigarettes Reduce Embryo to Placental Weight Ratios and Elicit Differential Gene Expression of Placental Growth, Invasion, and Antioxidant Response Pathways

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A rising 10% of women report using electronic cigarettes or e-cigarettes as a supposed safer substitute for traditional cigarettes during pregnancy. Cigarette use during pregnancy impairs embryo implantation, induces pre-term birth, and can result in fetal developmental abnormalities. Yet, few studies have examined how e-cigarettes impact pregnancy. Unlike cigarettes, e-cigarettes heat and aerosolize a base liquid consisting of propylene glycol and vegetable glycerine with additives such as nicotine and flavorings. During early pregnancy, the placenta develops after trophoblast cells invade and remodel the maternal artery network. Trophoblast cell invasion is promoted by the hormone peptide, adrenomedullin (ADM), and angiogenesis is induced by placental growth factor (PGF). As the placenta grows, reactive oxygen species can develop eliciting upregulation of antioxidants including the selenoprotein families: glutathione peroxidase (GPX) and thioredoxin reductase (TXNRD). Recent studies have shown e-cigarette liquids impaired *in vitro* trophoblast cell invasion; yet no studies have examined flavored e-cigarettes. Furthermore, the impact of e-cigarette use on placental development is unknown. We aimed to examine how e-cigarette liquid with flavoring can impact trophoblast cell gene expression and placental development. **We hypothesized that e-cigarette vapors with flavoring impair placental growth pathways and induce an antioxidant response.** To test this hypothesis, wildtype C57BL/6J animals were exposed to sham or e-cigarette vapors containing flavoring with and without nicotine (6 mg/mL) until day 12.5 of pregnancy. Concurrently, the same e-cigarette liquids were heated and condensed. These condensates were administered for 6 hours at a 1.5% concentration to the transformed trophoblast cell line, HTR8/SVneo. In the e-cigarette exposed animals, embryo to placental weight ratios were decreased significantly using one-way ANOVA with the student's t-test (n=76 fetal-placental pairs). Furthermore, in the absence of nicotine, GPX3 and GPX4 mRNA was significantly reduced in female placentas using the student's t-test (n=3 placentas). Thus, e-cigarette exposure impairs fetal-placental growth and can dampen the GPX antioxidant response in a sex-specific manner. In the trophoblast cells, placental growth regulator PGF, was upregulated while ADM was downregulated using the student's t-test (n=3 replicates). In the absence of nicotine, TXNRD1 was upregulated. Hence, e-cigarette condensates may promote trophoblast cell growth and the antioxidant response via PGF and TXNRD1, but impair ADM-driven trophoblast invasion. Therefore, e-cigarettes with flavoring reduce embryo to placental weight ratios and modulate genes critical for placental development and the antioxidant response. Future directions aim to measure *in situ* levels of reactive oxygen species upon e-cigarette exposure and identify how nicotine contributes to the placental antioxidant response. These studies suggest a measure of caution be observed when using these devices during pregnancy. This work was supported by NICHD 1K99HD10490001, R00HD104900 to MWM.