



HHS Public Access

Author manuscript

Environ Int. Author manuscript; available in PMC 2022 November 01.

Published in final edited form as:

Environ Int. 2022 November ; 169: 107552. doi:10.1016/j.envint.2022.107552.

Using follicular fluid metabolomics to investigate the association between air pollution and oocyte quality

Sueyoun Hwang^a, Robert B. Hood^a, Russ Hauser^{b,c}, Joel Schwartz^{b,c,d}, Francine Laden^{b,c,d}, Dean Jones^e, Donghai Liang^f, Audrey J. Gaskins^{a,*}

^aDepartment of Epidemiology, Emory University Rollins School of Public Health, Atlanta, GA, United States

^bDepartment of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, United States

^cDepartment of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, United States

^dChanning Division of Network Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, United States

^eDivision of Pulmonary, Allergy, & Critical Care Medicine, Emory University School of Medicine, Atlanta, GA, United States

^fGangarosa Department of Environmental Health, Emory University Rollins School of Public Health, Atlanta, GA, United States

Abstract

Background and aim: Our objective was to use metabolomics in a toxicological-relevant target tissue to gain insight into the biological processes that may underlie the negative association between air pollution exposure and oocyte quality.

Methods: Our study included 125 women undergoing *in vitro* fertilization at an academic fertility center in Massachusetts, US (2005–2015). A follicular fluid sample was collected during oocyte retrieval and untargeted metabolic profiling was conducted using liquid chromatography with ultra-high-resolution mass spectrometry and two chromatography columns (C18 and HILIC).

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

*Corresponding author at: Sc.D., 1518 Clifton Road, CNR 3017, Atlanta, GA 30322, United States. audrey.jane.gaskins@emory.edu (A.J. Gaskins).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Sueyoun Hwang: Formal analysis, Writing – original draft. **Robert B. Hood:** Validation, Methodology, Writing – review & editing. **Russ Hauser:** Data curation, Project administration, Funding acquisition, Writing – review & editing. **Joel Schwartz:** Resources, Writing – review & editing. **Francine Laden:** Resources, Writing – review & editing. **Dean Jones:** Data curation, Resources, Writing – review & editing. **Donghai Liang:** Formal analysis, Methodology, Supervision, Writing – review & editing. **Audrey J. Gaskins:** Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2022.107552>.

Daily exposure to nitrogen dioxide (NO₂), ozone, fine particulate matter, and black carbon was estimated at the women's residence using spatiotemporal models and averaged over the period of ovarian stimulation (2-weeks). Multivariable linear regression models were used to evaluate the associations between the air pollutants, number of mature oocytes, and metabolic feature intensities. A meet-in-the-middle approach was used to identify overlapping features and metabolic pathways.

Results: Of the air pollutants, NO₂ exposure had the largest number of overlapping metabolites (C18: 105; HILIC: 91) and biological pathways (C18: 3; HILIC: 6) with number of mature oocytes. Key pathways of overlap included vitamin D3 metabolism (both columns), bile acid biosynthesis (both columns), C21-steroid hormone metabolism (HILIC), androgen and estrogen metabolism (HILIC), vitamin A metabolism (HILIC), carnitine shuttle (HILIC), and prostaglandin formation (C18). Three overlapping metabolites were confirmed with level-1 or level-2 evidence. For example, hypoxanthine, a metabolite that protects against oxidant-induced cell injury, was positively associated with NO₂ exposure and negatively associated with number of mature oocytes. Minimal overlap was observed between the other pollutants and the number of mature oocytes.

Conclusions: Higher exposure to NO₂ during ovarian stimulation was associated with many metabolites and biologic pathways involved in endogenous vitamin metabolism, hormone synthesis, and oxidative stress that may mediate the observed associations with lower oocyte quality.

Keywords

Air pollution; Fertility; Metabolomics; Ovary; Assisted reproduction; Follicular fluid

1. Introduction

Recently, studies have suggested a negative association between exposure to ambient air pollution and reduced fertility. (Gaskins et al., 2021; Conforti et al., 2018; Frutos et al., 2015) While the association between air pollution and semen quality is well documented (Zhang et al., 2020), less is known about the harmful effect of periconception air pollution on female fertility, and more specifically ovarian function. Recent studies found evidence of ovarian dysfunction in mice following exposure to fine particulate matter (PM_{2.5}), a common air pollutant, shown by exacerbated ovarian oxidative stress and inflammation (Zhou et al., 2020) and apoptosis of ovarian granulosa cells and oocytes. (Liao et al., 2020) Two studies – the first among subfertile American women (Gaskins et al., 2019) and the second among subfertile Korean women (Kim et al., 2021) – have also documented an association between higher exposure to outdoor air pollution and lower ovarian reserve. While these human studies support the animal literature suggesting that air pollution has a specific detrimental effect on ovarian function, the potential biological mechanisms remain unclear.

Studies evaluating the association between air pollution and female fertility are often conducted among women utilizing assisted reproductive technology (ART) as it allows for the direct measure of markers of fertility (e.g., oocyte quality), can document important

early reproductive events (e.g., embryo development), and can define exact windows of air pollution exposure for critical stages of reproduction. Integrating metabolomic markers into these types of studies may also help us better understand the important mediators underlying the causal pathways between air pollution and human reproduction. A recent study of 200 women who underwent a fresh autologous ART cycle identified several pre-conception serum metabolic features and pathways as potential mediators of the negative association between air pollution and live birth. (Gaskins et al., 2021) While this study focused on serum metabolites, previous work has suggested that the primary target of air pollution may be the ovary. (Zhou et al., 2020; Liao et al., 2020) Therefore, studying the follicular fluid, which is the immediate microenvironment surrounding the oocyte and its constituents, (Dumesic et al., 2015) may represent the ideal matrix for measuring and identifying metabolites of interest. (Bracewell-Milnes et al., 2017) Measuring metabolites in the follicular fluid will also allow for the investigation of early biomarkers of maternal exposure to air pollution that may be unique to the ovary. Second, while our previous study focused on the clinically relevant outcome of live birth, a more temporally and biologically relevant outcome may be oocyte quality which is a critical component of female fertility and is often influenced by external factors including maternal exposure to air pollution. (Krisner, 2013).

Building on this previous research, our objective was to use untargeted high-resolution metabolomics to identify metabolites and pathways in the follicular fluid associated with periconceptional exposure to air pollution, including nitrogen dioxide (NO₂), black carbon (BC), PM_{2.5}, and ozone (O₃), and oocyte quality among a prospective cohort of women undergoing fresh autologous ART. Findings from this study will help improve our understanding of the biological relationship between ambient air pollution and female fertility.

2. Methods

2.1. Study design and participants

Women included in our analysis were a sub-set of participants in the Environment and Reproductive Health (EARTH) study (Messerlian et al., 2018), a prospective cohort designed to evaluate environmental and nutritional determinants of fertility among couples presenting for infertility treatment and evaluation at the Massachusetts General Hospital Fertility Center (2005–2019). The EARTH study was approved by the Human Studies Institutional Review Boards of the MGH and the Harvard T. H. Chan School of Public Health. In brief, 135 women from the EARTH study who underwent a fresh assisted reproductive technology (ART) cycle and provided a follicular fluid sample during oocyte retrieval were eligible for our analysis. We then excluded 10 women who did not provide a follicular fluid sample from their first dominant follicle to reduce variability due to this factor. This left 125 women for our analysis. A further description of our study flow is provided in Supplemental Fig. 1.

2.2. Air pollution assessment

At enrollment, all women in our study provided their residential address for reimbursement purposes. These addresses were geocoded using ArcGIS and linked to several existing

spatio-temporal models to derive daily ambient air pollution exposures at the woman's address starting 3 months prior to the date of oocyte retrieval. Daily PM_{2.5} and NO₂ concentrations were modeled at a 1 km² resolution using satellite remote sensing data in combination with land use terms. (Kloog et al., 2014; Lee and Koutrakis.) Daily O₃ concentrations were also modelled at a 1 km² resolution using chemical transport models, O₃ vertical profiles, meteorological variables, and other atmospheric compounds. (Di et al., 2017) Daily BC exposure was estimated at the home address using support vector machine regression models based on ambient measurements collected across New England as well as several spatial and temporal predictors. (Abu Awad et al., 2017) In this analysis, we focused on average ambient air pollution exposure in the two weeks prior to oocyte retrieval as it represents both a clinically (e.g. during controlled ovarian stimulation) and biologically (e.g. during the final stages of oocyte maturation) relevant time window.

2.3. Outcome assessment

Women in our study underwent one of three controlled ovarian stimulation protocols as clinically indicated: luteal-phase gonadotropin hormone-releasing hormone (GnRH) agonist, GnRH-antagonist protocol, or a follicular phase GnRH-agonist protocol. During gonadotropin stimulation, women were monitored to ensure follicular development including serum estradiol, follicle size measurements and counts, and endometrial thickness. Once 3 or more lead follicles (≥ 16 mm in diameter) were visualized and the estradiol level was >600 pmol/L, human chorionic gonadotropin (hCG) was administered to induce oocyte maturation and 35–37 h later oocyte retrieval was performed using a transvaginal ultrasound guided aspiration. During oocyte retrieval, a follicular fluid sample was taken from women's first three follicles with a 16 G needle. Each sample was collected in a separate tube prepared with 1 mL of flushing media. Once the oocytes were removed, the follicular fluid was centrifuged to separate the supernatant and pellet. The resulting aliquots were then stored at –80 °C. Embryologists classified the retrieved oocytes as germinal vesicle, metaphase I, metaphase II (MII) or degenerated. Total oocyte yield was defined as the sum of all oocytes retrieved regardless of type. Mature oocyte yield was the sum of all MII oocytes.

2.4. Metabolomic assessment

Follicular fluid supernatant samples were shipped overnight, on dry ice, to Emory University for metabolomics analysis. Once received, samples were randomized prior to analysis to minimize batch effects. We utilized a standardized workflow to analyze the samples (Supplemental Fig. 1). Follicular fluid samples were analyzed using liquid chromatography with high resolution mass spectrometry (LC-HRMS; Dionex Ultimate 3000 RSLCnano; Thermo Orbitrap Fusion), which has been described in greater detail elsewhere. (xxxx) To facilitate greater feature detection, two chromatography columns were used: the C18 hydrophobic chromatography column (Higgins Targa C18; 2.1 × 50 mm²; 3 μm particle size) with negative electrospray ionization (ESI) (–30 kV) and the hydrophilic interaction chromatography (HILIC) column (Waters XBridge BEH Amide XP HILIC; 2.1 × 50 mm²; 2.6 μm particle size) with positive ESI (3.5 kV). The sheath and auxiliary gases were set at 30 (arbitrary units) and 5 (arbitrary units) for the C18 column, respectively. The mobile phase for the C18 column included 60 % water, 35 % acetonitrile, 5 % 10 mM

ammonium acetate and was linearly increased to 0 % water, 95 % acetonitrile, and 5 % 10 mM ammonium acetate at 3 min and was held for 2 min. The main adducts formed in the C18 column included: $M-H$, $M+Cl$, $M+CH_3CO_2$, $M+HCO_2$, $M-2H$, and $2M-3H$. In the HILIC column, the sheath and auxiliary gases were set at 45 (arbitrary units) and 25 (arbitrary units), respectively. The mobile phase for the HILIC column included 22.5 % water, 75 % acetonitrile, and 2.5 % 2 % formic acid for the first 1.5 min and was linearly increased to 75 % water, 22.5 % acetonitrile, and 2.5 % formic acid at 4 min and was held for 1 min. The main adducts formed in the HILIC column included $M+H$, $M+2Na-H$, $M+Na$, $M-H_2O+H$, $M+K$, $M+3H$, $M-2H_2O+H$, $2M+H$, $2M-H$, and $2M+ACN+H$. For both columns, the flow rate was 0.35 mL/minute for the first minute and increased to 0.4 mL/minute for the last 4 min. The resolution of the HRMS was set at 120 k and the capillary and source temperatures were set at 275 °C and 45 °C, respectively. The m/z collection ranged from 85 to 1275. We used two quality control samples, NIST 1950 [18] and pooled human plasma (Equitech Bio), which are described elsewhere. (Go et al., 2015) Briefly, in the experimental phase, the pooled human plasma assisted with batch correction. The NIST 1950 samples assisted with signal intensity normalization across batches relative to a calibrated reference. Inclusion of the NIST 1950 samples allowed for better intra- and inter-laboratory comparison. We used ProteoWizard to convert raw data files to.mzML files using apLCMS and xMSanalyzer. These are common packages that have been described elsewhere (Chambers et al., 2012; Uppal et al., 2013; Yu et al., 2009) but briefly they process raw data files for peak extraction and ion intensity quantification. Unique features were characterized based on mass-to-charge ratio (m/z), retention time, and ion intensity. To filter out the noise signals and optimize the metabolomics data quality, only metabolic features detected in > 10 % of the follicular fluid samples with median coefficient of variation (CV) < 30 % and Pearson correlation > 0.7 among technical triplicates, were included in further analyses. Following quality assessment, the median intensity was taken across replicate samples and these intensities were natural log transformed for analysis.

2.5. Statistical analysis

To assess the overall association between each of the air pollutants and number of mature oocytes retrieved, we used a Poisson model adjusting for age (continuous), body mass index (BMI, continuous), smoking status (categorical: never, ever), education (categorical: < college, college graduates, graduate degree), average temperature (continuous), and protocol (categorical: luteal phase agonist, flare or antagonist). We analyzed the associations between air pollutant exposure in the two weeks prior to oocyte retrieval and metabolic features in the follicular fluid using multivariable generalized linear models (GLM) adjusted for age (continuous), body mass index (BMI, continuous), smoking status (categorical: never, ever), education (categorical: < college, college graduates, graduate degree), and average temperature (continuous). Separate models were conducted for each metabolic feature detected in each chromatography chrome (i.e., C18 column with negative ESI and HILIC column with positive ESI). Similar models adjusted for age, BMI, smoking status, education, protocol (categorical: luteal phase agonist, flare or antagonist) were used to examine the association between total number of MII oocytes retrieved and metabolic features. In the GLMs, the outcome was the natural log transformation of the intensity of the detected features and the main independent variable of interest was either air

pollutant exposure or total number of MII oocytes retrieved. All models utilized a Gaussian distribution. Multiple comparison correction was conducted using the Benjamini-Hochberg false discovery rate (FDR_{B-H}) procedure, a widely used procedure in MWAS studies, at a 5 % false positive threshold. Analyses were completed in R/R studio (v 4.2.0, R Foundation for Statistical Computing, Vienna, Austria).

We conducted pathway enrichment analysis using all the metabolic features significant at $p < 0.05$ by utilizing mummichog (v. 1.0.10), a bioinformatics platform that infers and categorizes functional biological activity directly from mass spectrometry output, without prior metabolite validation. (Li et al., 2013;9(7):e1003123.) An adjusted p-value for each pathway was calculated from resampling the reference input file in mummichog using a gamma distribution, which penalizes pathways with fewer reference hits, and assigning greater significance to pathways with more reference hits. (Li et al., 2013;9(7):e1003123.) We conducted pathway analysis separately for each of the air pollutants and for each chromatography column. Heat maps were used to display the associations with the top metabolic pathways. We used an in-house database of previously confirmed metabolites to annotate the significant features identified in our analyses (Liu et al., 2020; Liu et al., 2021). This annotation was based on a comparison of adduct, m/z , retention time, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) spectra to analytical standards or database spectra to confirm level-1 and level-2 evidence based on the Metabolomics Standards Initiative criteria. (Go et al., 2015; Goodacre et al., 2007) Level-1 evidence is defined as match with an authentic standard while level-2 evidence is defined as a match with database spectra; of the two, level-1 evidence is considered stronger evidence of an identification.

3. Results

The 125 women included in our analysis had a mean age of 34.7 years and BMI of 24.0 kg/m^2 (Supplemental Table 1). The majority were white (86 %), never smokers (77 %), with a college degree or higher (93 %). Most women had been diagnosed with unexplained infertility (43 %) and were treated with a luteal phase agonist protocol (72 %). The mean (standard deviation) number of mature oocytes retrieved was 9.7 (4.8). The median (25th, 75th percentile) exposure to air pollution in the 2 weeks prior to oocyte retrieval was 22.3 (15.3, 36.0) ppb for NO_2 , 32.2 (26.1, 42.0) ppb for O_3 , 8.4 (7.3, 10.1) $\mu\text{g}/\text{m}^3$ for $\text{PM}_{2.5}$, and 0.5 (0.4, 0.7) $\mu\text{g}/\text{m}^3$ for BC (Supplemental Table 2). The air pollutants were weakly to moderately correlated with one another ($\rho = 0.07$ for NO_2 and $\text{PM}_{2.5}$ to 0.39 for NO_2 and BC) (Supplemental Table 3). There was little association between average exposure to air pollution during the two weeks of ovarian stimulation and number of mature oocytes retrieved. For example, the percent change in number of mature oocytes retrieved per IQR increase was 1.4 % (95 % CI -4.5, 7.4) for NO_2 , 6.6 % (95 % CI 1.1, 12.1) for O_3 , 2.4 % (95 % CI -3.2, 8.3) for $\text{PM}_{2.5}$, and 8.8 % (95 % CI 2.5, 15.3) for BC.

From the C18 and HILIC chromatography columns, 14,394 and 17,161 metabolic features were extracted from the follicular fluid, respectively. Across the two columns, thousands of metabolic features were associated with each air pollutant at a raw p-value < 0.05 (Table 1); however, after FDR correction (< 0.20), only higher exposure to NO_2 and O_3 in the

two weeks prior to oocyte retrieval was associated with 96 and 47 feature(s), respectively. Using the features with raw p-value < 0.05 as inputs, there were 5, 1, 2, and 1 metabolic pathways in the C18 chromatography column associated with exposure to NO₂, O₃, PM_{2.5}, and BC, respectively (Fig. 1A). In the HILIC chromatography column, 7, 5, 6, and 5 metabolic pathways were associated with NO₂, O₃, PM_{2.5}, and BC exposure, respectively (Fig. 1B). Only four pathways were associated with more than one pollutant- Vitamin D3 (cholecalciferol) metabolism (NO₂ and PM_{2.5}), Vitamin A (retinol) metabolism (NO₂ and PM_{2.5}), C21-steroid hormone biosynthesis and metabolism (NO₂ and BC), and Bile acid biosynthesis (NO₂, O₃ and BC).

There were 897 and 1046 metabolic features in the follicular fluid that were associated with total mature oocytes at a raw p-value < 0.05 (Table 1). After FDR correction (P < 0.20), only three features remained significant. Using the features with raw p-value < 0.05 as inputs into the pathway analysis, 7 and 9 pathways were significantly associated with total mature oocytes in the C18 and HILIC chromatography columns, respectively (Fig. 1A & B). Top hits in the C18 chromatography column included Bile acid biosynthesis, Vitamin D3 (cholecalciferol) metabolism, Prostaglandin formation from dihomo gamma-linoleic acid, Urea cycle/amino group metabolism, Ascorbate (Vitamin C) and aldarate metabolism, Polyunsaturated fatty acid biosynthesis, and Caffeine metabolism. Top hits in the HILIC chromatography column included Carnitine shuttle, C21-steroid hormone biosynthesis and metabolism, Vitamin D3 (cholecalciferol) metabolism, Vitamin A (retinol) metabolism, Tyrosine metabolism, Androgen and estrogen biosynthesis and metabolism, Bile acid biosynthesis, Propanoate metabolism, and Squalene and cholesterol biosynthesis.

We found 105 and 91 overlapped metabolic features with raw p-value < 0.05, associated with both NO₂ exposure and number of mature oocytes retrieved from the C18 and HILIC chromatography columns. Most of the pathways that were associated with the air pollutants and mature oocytes were non-overlapping in both chromatography columns (Fig. 1A & B). However, there were three pathways in the C18 chromatography column and six pathways in the HILIC chromatography column that were shared between at least one air pollutant exposure and number of mature oocytes retrieved. In C18 chromatography column, metabolites in the Prostaglandin formation from dihomo gamma-linoleic acid, Vitamin D3 (cholecalciferol) metabolism, Bile acid biosynthesis were altered with higher exposure to NO₂ and varied according to number of mature oocytes. In HILIC chromatography column, dysregulation of Vitamin D3 (cholecalciferol) metabolism and Vitamin A (retinol) metabolism was shared between NO₂ and PM_{2.5} exposure and mature oocytes and a dysregulation in the C21-steroid hormone biosynthesis and metabolism was shared between NO₂ and BC exposure and mature oocytes. Androgen and estrogen biosynthesis and metabolism and Carnitine shuttle were shared between NO₂ exposure and mature oocytes. Bile acid biosynthesis were shared among NO₂, O₃, BC exposure and mature oocytes.

We further confirmed 4 metabolites with level-1 evidence and 4 metabolites with level-2 evidence (Table 2). Metabolites that were associated with both NO₂ exposure and number of mature oocytes included hypoxanthine, d-lactose, and caffeine. While the metabolites that were associated with BC or PM_{2.5} were negatively associated with the pollutants – being higher among women with lower exposure to air pollution, we found the opposite pattern

for NO₂ as the annotated metabolites were higher among women with higher exposure to air pollution.

4. Discussion

We applied untargeted high-resolution metabolomics to follicular fluid, a toxicologically relevant target tissue, to lend insight into the potential biological mechanisms underlying the relationship between ambient air pollution exposure and oocyte quality among women undergoing ART. Of the air pollutants examined, NO₂, which tends to be a marker of vehicle emissions, had the largest number of overlapping metabolites and metabolic pathways with the number of mature oocytes retrieved while the other air pollutants, O₃, PM_{2.5}, and BC had limited overlap. Our study provides novel mechanistic insight into the potential biological pathways such as endogenous vitamin metabolism, hormone synthesis, and oxidative stress and the specific metabolites, such as hypoxanthine, that may be mediating the negative association between NO₂ exposure and lower oocyte quality in women.

The finding that NO₂ exposure had the largest number of overlapping metabolites and pathways with mature oocyte yield is in line with previous epidemiological studies suggesting that women with higher exposure to traffic-related air pollution have lower fertility as measured by high incidence of infertility, (Mahalingaiah et al., 2016) longer time to pregnancy, (Wesselink et al., 2020) decreased success with ART, (Gaskins et al., 2019) and higher risk of pregnancy loss. (Kioumourtoglou et al., 2019) Moreover, it suggests that compromised oocyte quality could be a primary mediator. An experimental study in mice found a significant reduction in the number of ovarian antral follicles following traffic-generated PM exposure. (Veras et al., 2009) PM-induced ovarian damages are also demonstrated as an inflammatory response in ovarian tissues, ovarian oxidative stress, apoptosis, and abnormal ultrastructural alterations in mice. (Gai et al., 2017) The findings from the animal literature can thus provide biological plausibility to why NO₂, which is another pollutant associated with traffic emissions, was the strongest pollutant associated with mature oocyte yield in our study.

Three key pathways that were shared between NO₂ exposure and the number of mature oocytes retrieved included vitamin D3 metabolism, vitamin A metabolism, and bile acid biosynthesis. Vitamin D and A have long been implicated in human reproduction. Vitamin D signaling is directly involved in the expression of the anti-Mullerian hormone (AMH), which is produced by the ovarian granulosa cells and known to play a role in the regulation of follicular recruitment and selection. Therefore, vitamin D deficiency in females may contribute to impairment in ovarian physiology via disrupted AMH signaling. (Luk et al., 2012) Given that enzymes known to be involved in retinoid synthesis are found in the ovary, it is plausible that vitamin A deficiency can lead to the deterioration of oocyte quality. (Clagett-Dame and DeLuca, 2002) Emerging evidence also suggests that air pollution may directly (through reduced UVB exposure) and indirectly (through less time spent outdoors) lessen the cutaneous production of vitamin D3 (Mousavi et al., 2019) and reduce levels of the vitamin A precursor, β carotene (a potent antioxidant), in the body. (Bernard et al., 1998) It is also interesting that bile acid biosynthesis was implicated as the absorption of lipid-soluble vitamins (such as vitamins D and A) from the diet requires bile acids and high

levels of these vitamins may also repress bile acid synthesis to protect against potentially toxic levels of lipid-soluble vitamins in the diet. (Schmidt et al., 2010) Traffic-related air pollution has been known to alter bile acid homeostasis (Dutta et al., 2022) and previous studies on air pollution and the blood metabolome (Gaskins et al., 2021; Liang et al., 2018; Liang et al., 2019) have also found bile acid biosynthesis to be commonly dysregulated pathway with higher exposure to air pollution.

Other overlapping pathways associated with both NO₂ and oocyte quality included C21-steroid hormone metabolism, androgen and estrogen metabolism, and prostaglandin formation. Steroid hormones are considered critical elements in reproductive outcomes and the composition of these hormones in the follicular environment is an important determinant of oocyte quality. (Carpintero et al., 2014) Environmental pollutants are known to interfere with steroid hormone metabolism through disruption of hydroxysteroid dehydrogenases, a group of steroidogenic enzymes, resulting in impaired reproductive functions. (Ye et al., 2014) Diesel exhaust, in particular, contains a variety of substances including polycyclic aromatic hydrocarbons with documented estrogenic, anti-estrogenic, and anti-androgenic properties that can affect gonadal steroidogenesis and gametogenesis. (Rekhadevi et al., 2014; Archibong et al., 2012; Takeda et al., 2004; Plunk and Richards, 2020) The last overlapping pathway that might also mediate the association between NO₂ and oocyte quality is the carnitine shuttle. L-carnitine plays an important role in female reproduction, more specifically, oocyte development and quality enhancement. It acts as an antioxidant by promoting β-oxidation in oocytes, attenuating oxidative damage, and preventing apoptosis. (Agarwal et al., 2018; Li et al., 2021) Metabolites in carnitine shuttle metabolism such as acyl-carnitines have also been consistently reported to be affected by traffic-related air pollutants most notably in association with NO₂. (van Veldhoven et al., 2019).

Although not all metabolites reached level-1 evidence, we are confident in the level-2 metabolites and considering the exploratory nature of this analysis, we believe some of these metabolites are still worth mentioning. For instance, hypoxanthine, a purine derivative that protects against oxidant-induced cell injury, (Virág and Szabó, 2001) appears to be the most intuitive potential mediator as it was increased with higher exposure to NO₂ and was increased among women with fewer mature oocytes retrieved. Corroborating this finding include multiple studies which found this specific metabolite to be associated with air pollution in the plasma metabolome (Vlaanderen et al., 2017; Nassan et al., 2021; Chen et al., 2019) and multiple experimental studies which showed that hypoxanthine plays a critical role in inhibiting the nuclear maturation of oocytes. (Downs, 1997; Downs et al., 1985) While we also observed two other metabolites, d-lactose and caffeine, to be associated with both NO₂ exposure and number of mature oocytes retrieved, their role as potential mediators was less intuitive based on previous research and biological hypotheses.

In a previous paper from our group, we identified 13 metabolic pathways in serum preconception samples that were significantly associated with NO₂ exposure and probability of live birth following ART. (Gaskins et al., 2021) Interestingly, none of these pathways overlapped with the follicular fluid pathways associated with air pollution and mature oocyte yield. While three follicular fluid pathways associated with mature oocyte yield-bile acid biosynthesis, urea cycle/amino group metabolism, and ascorbate (vitamin C) and

aldarate metabolism-were also found in the serum to be associated with probability of live birth following ART, almost none of the serum pathways associated with NO₂ exposure overlapped with the follicular fluid pathways associated with NO₂ exposure. This is likely due to a combination of several factors. First, the follicular fluid is a distinct biological fluid from blood and may represent a more toxicologically relevant biofluid for investigating the impacts of air pollution on ovarian function. (Qasemi and Amidi, 2020) Second, NO₂ exposure may have a specific negative impact on the ovary, (Gregoire et al., 2021; La Marca et al., 2020) which may be better captured by studying follicular fluid as opposed to blood.

While the findings from this study expand our understanding of the biological mechanism underlying the negative association between air pollution exposure and oocyte quality, they should also be considered in the context of its limitations. First, given the large number of metabolic features we identified and multiple comparisons made between different air pollutants, there is an increased probability of false-positive and Type 1 errors. Although we reported the number of significant metabolic features at different levels of p-value including FDR_{B-H} correction, we had to use a cut-off of raw p-value < 0.05 for our pathway analyses to allow for meaningful interpretation. Considering our small sample size, we used less stringent criteria for statistical significance to decrease the false-negative rates. Second, a proxy to estimate ambient air pollution exposure using participants' residential addresses may not be perfect, which could decrease the precision of our effect estimates. However, the prospective design of this study made it less likely for this uncertainty in exposure assessment to be influenced by our outcome. Third, since our sample is subfertile with most being white and highly educated, our results may not be generalizable to the broader population. The women in our study's exposure to air pollution also tended to be low. Therefore, it is possible we may have underestimated or missed associations that could be present in other, more highly polluted regions.

In summary, we successfully identified metabolites and pathways in the follicular fluid that are overlapping between periconceptional exposure to air pollution and oocyte quality using untargeted high-resolution metabolomics and a 'meet-in-the-middle' approach. These results provide valuable information to the investigation of how air pollutants, in particular those due to traffic, may negatively impact oocyte quality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank all members of the EARTH study team, specifically research nurses Jennifer B. Ford and Myra G. Keller, senior research staff Ramace Dadd, the physicians and staff at Massachusetts General Hospital Fertility Center, and all the EARTH study participants.

Funding:

Supported by grants ES009718, ES022955, ES000002, and ES026648 from the National Institute of Environmental Health Sciences (NIEHS). The study was also supported by HERCULES through the NIEHS (P30 ES019776). This publication was also made possible by U.S. Environmental Protection Agency (U.S. EPA): RD-834798 and

RD-83587201. Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the U.S. EPA. Further, U.S. EPA does not endorse the purchase of any commercial products or services mentioned in the publication.

Data availability

Data will be made available on request.

References

- Abu Awad Y, Koutrakis P, Coull BA, Schwartz J, 2017. A spatio-temporal prediction model based on support vector machine regression: Ambient Black Carbon in three New England States. *Environ Res.* 159, 427–434. 10.1016/j.envres.2017.08.039. [PubMed: 28858756]
- Agarwal A, Sengupta P, Durairajanayagam D, 2018. Role of L-carnitine in female infertility. *Reprod Biol Endocrinol.* 16 (1), 5. 10.1186/s12958-018-0323-4. [PubMed: 29373970]
- Archibong AE, Ramesh A, Inyang F, Niaz MS, Hood DB, Kopsombut P, 2012. Endocrine disruptive actions of inhaled benzo(a)pyrene on ovarian function and fetal survival in fisher F-344 adult rats. *Reprod Toxicol.* 34 (4), 635–643. 10.1016/j.reprotox.2012.09.003. [PubMed: 23059060]
- Bernard N, Saintot M, Astre C, Gerber M, 1998. Personal Exposure to Nitrogen Dioxide Pollution and Effect on Plasma Antioxidants. *Arch Environ Health Int J.* 53 (2), 122–128. 10.1080/00039896.1998.10545973.
- Bracewell-Milnes T, Saso S, Abdalla H, et al. Metabolomics as a tool to identify biomarkers to predict and improve outcomes in reproductive medicine: a systematic review. *Hum Reprod Update.* 2017;23(6):723–736. doi:10.1093/humupd/dmx023. [PubMed: 29069503]
- Carpintero N, Mangas C, Rioja R, Suárez O, Varea C, 2014. Follicular steroid hormones as markers of oocyte quality and oocyte development potential. *J Hum Reprod Sci.* 7 (3), 187. 10.4103/0974-1208.142479. [PubMed: 25395744]
- Chambers MC, Maclean B, Burke R, Amodei D, Ruderman DL, Neumann S, Gatto L, Fischer B, Pratt B, Egertson J, Hoff K, Kessner D, Tasman N, Shulman N, Frewen B, Baker TA, Brusniak M-Y, Paulse C, Creasy D, Flashner L, Kani K, Moulding C, Seymour SL, Nuwaysir LM, Lefebvre B, Kuhlmann F, Roark J, Rainer P, Detlev S, Hemenway T, Huhmer A, Langridge J, Connolly B, Chadick T, Holly K, Eckels J, Deutsch EW, Moritz RL, Katz JE, Agus DB, MacCoss M, Tabb DL, Mallick P, 2012. A cross-platform toolkit for mass spectrometry and proteomics. *Nat Biotechnol.* 30 (10), 918–920. [PubMed: 23051804]
- Chen C, Li H, Niu Y, et al. Impact of short-term exposure to fine particulate matter air pollution on urinary metabolome: A randomized, double-blind, crossover trial. *Environ Int.* 2019;130:104878. doi:10.1016/j.envint.2019.05.072. [PubMed: 31200160]
- Clagett-Dame M, DeLuca HF, 2002. THE ROLE OF VITAMIN A IN MAMMALIAN REPRODUCTION AND EMBRYONIC DEVELOPMENT. *Annu Rev Nutr.* 22 (1), 347–381. 10.1146/annurev.nutr.22.010402.102745E. [PubMed: 12055350]
- Conforti A, Mascia M, Cioffi G, De Angelis C, Coppola G, De Rosa P, Pivonello R, Alviggi C, De Placido G, 2018. Air pollution and female fertility: a systematic review of literature. *Reprod Biol Endocrinol.* 16 (1) 10.1186/s12958-018-0433-z.
- Di Q, Rowland S, Koutrakis P, Schwartz J, 2017. A hybrid model for spatially and temporally resolved ozone exposures in the continental United States. *J Air Waste Manag Assoc.* 67 (1), 39–52. 10.1080/10962247.2016.1200159. [PubMed: 27332675]
- Downs SM, 1997. Hypoxanthine Regulation of Oocyte Maturation in the Mouse: Insights Using Hypoxanthine Phosphoribosyltransferase-Deficient Animals I. *Biol Reprod.* 57 (1), 54–62. 10.1095/biolreprod57.1.54. [PubMed: 9209080]
- Downs SM, Coleman DL, Ward-Bailey PF, Eppig JJ, 1985. Hypoxanthine is the principal inhibitor of murine oocyte maturation in a low molecular weight fraction of porcine follicular fluid. *Proc Natl Acad Sci.* 82 (2), 454–458. 10.1073/pnas.82.2.454. [PubMed: 2982158]
- Dumesic DA, Meldrum DR, Katz-Jaffe MG, Krisher RL, Schoolcraft WB, 2015. Oocyte environment: follicular fluid and cumulus cells are critical for oocyte health. *Fertil Steril.* 103 (2), 303–316. 10.1016/j.fertnstert.2014.11.015. [PubMed: 25497448]

- Dutta M, Weigel KM, Patten KT, Valenzuela AE, Wallis C, Bein KJ, Wexler AS, Lein PJ, Cui JY, 2022. Chronic exposure to ambient traffic-related air pollution (TRAP) alters gut microbial abundance and bile acid metabolism in a transgenic rat model of Alzheimer's disease. *Toxicol Rep.* 9, 432–444. [PubMed: 35310146]
- Frutos V, González-Comadrán M, Solà I, Jacquemin B, Carreras R, Checa Vizcaíno MA, 2015. Impact of air pollution on fertility: a systematic review. *Gynecol Endocrinol.* 31 (1), 7–13. 10.3109/09513590.2014.958992. [PubMed: 25212280]
- Gai HF, An JX, Qian XY, Wei YJ, Williams JP, Gao GL, 2017. Ovarian Damages Produced by Aerosolized Fine Particulate Matter (PM2.5) Pollution in Mice: Possible Protective Medications and Mechanisms. *Chin Med J (Engl).* 130 (12), 1400–1410. 10.4103/0366-6999.207472. [PubMed: 28584201]
- Gaskins AJ, Fong KC, Abu Awad Y, et al. Time-Varying Exposure to Air Pollution and Outcomes of *in Vitro* Fertilization among Couples from a Fertility Clinic. *Environ Health Perspect.* 2019;127(7):077002. doi:10.1289/EHP4601.
- Gaskins AJ, Tang Z, Hood RB, et al. Periconception air pollution, metabolomic biomarkers, and fertility among women undergoing assisted reproduction. *Environ Int.* 2021;155:106666. doi:10.1016/j.envint.2021.106666. [PubMed: 34116378]
- Gaskins AJ, Mínguez-Alarcón L, Fong KC, Abdelmessih S, Coull BA, Chavarro JE, Schwartz J, Kloog I, Souter I, Hauser R, Laden F, 2019. Exposure to Fine Particulate Matter and Ovarian Reserve Among Women from a Fertility Clinic. *Epidemiology.* 30 (4), 486–491. [PubMed: 31162281]
- Go Y-M, Walker DI, Liang Y, Uppal K, Soltow QA, Tran ViLinh, Strobel F, Quyyumi AA, Ziegler TR, Pennell KD, Miller GW, Jones DP, 2015. Reference Standardization for Mass Spectrometry and High-resolution Metabolomics Applications to Exposome Research. *Toxicol Sci.* 148 (2), 531–543. [PubMed: 26358001]
- Goodacre R, Broadhurst D, Smilde AK, Kristal BS, Baker JD, Beger R, Bessant C, Connor S, Capuani G, Craig A, Ebbels T, Kell DB, Manetti C, Newton J, Paternostro G, Somorjai R, Sjöström M, Trygg J, Wulfert F, 2007. Proposed minimum reporting standards for data analysis in metabolomics. *Metabolomics.* 3 (3), 231–241.
- Gregoire AM, Upson K, Niehoff NM, et al. Outdoor air pollution and anti-Müllerian hormone concentrations in the Sister Study. *Environ Epidemiol.* 2021;5(5):e163. doi: 10.1097/EE9.000000000000163. [PubMed: 34934887]
- Hood RB, Liang D, Tan Y, Ford J, Souter I, Jones D, Hauser R, Gaskins AJ. Characterizing the follicular fluid metabolome: quantifying the correlation across follicles and differences with the serum metabolome. *Fertil Steril.* In Press.
- Kim H, Choe S-A, Kim O-J, Kim S-Y, Kim S, Im C, Kim YS, Yoon TK, 2021. Outdoor air pollution and diminished ovarian reserve among infertile Korean women. *Environ Health Prev Med.* 26 (1) 10.1186/s12199-021-00942-4.
- Kioumourtzoglou MA, Raz R, Wilson A, et al. Traffic-related Air Pollution and Pregnancy Loss: *Epidemiology.* 2019;30(1):4–10. doi:10.1097/EDE.0000000000000918. [PubMed: 30199416]
- Kloog I, Chudnovsky AA, Just AC, Nordio F, Koutrakis P, Coull BA, Lyapustin A, Wang Y, Schwartz J, 2014. A new hybrid spatio-temporal model for estimating daily multi-year PM2.5 concentrations across northeastern USA using high resolution aerosol optical depth data. *Atmos Environ.* 95, 581–590.
- Krisher RL, 2013. In Vivo and In Vitro Environmental Effects on Mammalian Oocyte Quality. *Annu Rev Anim Biosci.* 1 (1), 393–417. 10.1146/annurev-animal-031412-103647. [PubMed: 25387025]
- La Marca A, Spaggiari G, Domenici D, et al. Elevated levels of nitrous dioxide are associated with lower AMH levels: a real-world analysis. *Hum Reprod.* 2020;35(11): 2589–2597. doi:10.1093/humrep/deaa214. [PubMed: 32951044]
- Lee HJ, Koutrakis P. Daily Ambient NO₂ Concentration Predictions Using Satellite Ozone Monitoring Instrument NO₂ Data and Land Use Regression. *Environ Sci Technol.* Published online February 4, 2014:140204134232009. doi:10.1021/es404845f.

- Li S, Park Y, Duraisingham S, et al. Predicting Network Activity from High Throughput Metabolomics. Ouzounis CA, ed. *PLoS Comput Biol.* 2013;9(7):e1003123. doi: 10.1371/journal.pcbi.1003123. [PubMed: 23861661]
- Li J, Liu L, Weng J, Yin T, Yang J, Feng HL, 2021. Biological roles of L-carnitine in oocyte and early embryo development. *Mol Reprod Dev.* 88 (10), 673–685. 10.1002/mrd.23542. [PubMed: 34618389]
- Liang D, Moutinho JL, Golan R, Yu T, Ladva CN, Niedzwiecki M, Walker DI, Sarnat SE, Chang HH, Greenwald R, Jones DP, Russell AG, Sarnat JA, 2018. Use of high-resolution metabolomics for the identification of metabolic signals associated with traffic-related air pollution. *Environ Int.* 120, 145–154. [PubMed: 30092452]
- Liang D, Ladva CN, Golan R, Yu T, Walker DI, Sarnat SE, Greenwald R, Uppal K, Tran ViLinh, Jones DP, Russell AG, Sarnat JA, 2019. Perturbations of the arginine metabolome following exposures to traffic-related air pollution in a panel of commuters with and without asthma. *Environ Int.* 127, 503–513. [PubMed: 30981021]
- Liao B-Q, Liu C-B, Xie S-J, Liu Y.u., Deng Y-B, He S-W, Fu X-P, Fu B-B, Wang Y-L, Chen M-H, Lin Y-H, Li F-P, Xie X.i., Hong X-R, Wang H-L, 2020. Effects of fine particulate matter (PM_{2.5}) on ovarian function and embryo quality in mice. *Environ Int.* 135, 105338. [PubMed: 31841806]
- Liu KH, Nellis M, Uppal K, Ma C, Tran ViLinh, Liang Y, Walker DI, Jones DP, 2020. Reference Standardization for Quantification and Harmonization of Large-Scale Metabolomics. *Anal Chem.* 92 (13), 8836–8844. [PubMed: 32490663]
- Liu KH, Lee CM, Singer G, Bais P, Castellanos F, Woodworth MH, Ziegler TR, Kraft CS, Miller GW, Li S, Go Y-M, Morgan ET, Jones DP, 2021. Large scale enzyme based xenobiotic identification for exposomics. *Nat Commun.* 12 (1) 10.1038/s41467-021-25698-x.
- Luk J, Torrealday S, Neal Perry G, Pal L, 2012. Relevance of vitamin D in reproduction. *Hum Reprod.* 27 (10), 3015–3027. 10.1093/humrep/des248. [PubMed: 22824625]
- Mahalingaiah S, Hart JE, Laden F, Farland LV, Hewlett MM, Chavarro J, Aschengrau A, Missmer SA, 2016. Adult air pollution exposure and risk of infertility in the Nurses' Health Study II. *Hum Reprod.* 31 (3), 638–647. [PubMed: 26724803]
- Messerlian C, Williams PL, Ford JB, et al. The Environment and Reproductive Health (EARTH) Study: a prospective preconception cohort. *Hum Reprod Open.* 2018;2018 (2). doi:10.1093/hropen/hoy001.
- Mousavi SE, Amini H, Heydarpour P, Amini Chermahini F, Godderis L, 2019. Air pollution, environmental chemicals, and smoking may trigger vitamin D deficiency: Evidence and potential mechanisms. *Environ Int.* 122, 67–90. 10.1016/j.envint.2018.11.052. [PubMed: 30509511]
- Nassan FL, Kelly RS, Kosheleva A, Koutrakis P, Vokonas PS, Lasky-Su JA, Schwartz JD, 2021. Metabolomic signatures of the long-term exposure to air pollution and temperature. *Environ Health.* 20 (1) 10.1186/s12940-020-00683-x.
- Plunk EC, Richards SM, 2020. Endocrine-Disrupting Air Pollutants and Their Effects on the Hypothalamus-Pituitary-Gonadal Axis. *Int J Mol Sci.* 21 (23), 9191. 10.3390/ijms21239191.
- Qasemi M, Amidi F, 2020. Extracellular microRNA profiling in human follicular fluid: new biomarkers in female reproductive potential. *J Assist Reprod Genet* 37 (8), 1769–1780. [PubMed: 32642870]
- Rekhadevi PV, Diggs DL, Huderson AC, Harris KL, Archibong AE, Ramesh A, 2014. Metabolism of the environmental toxicant benzo(a)pyrene by subcellular fractions of human ovary. *Hum Exp Toxicol.* 33 (2), 196–202. 10.1177/0960327113489050. [PubMed: 23703818]
- Schmidt DR, Holmstrom SR, Fon Tacer K, Bookout AL, Kliewer SA, Mangelsdorf DJ, 2010. Regulation of Bile Acid Synthesis by Fat-soluble Vitamins A and D. *J Biol Chem.* 285 (19), 14486–14494. 10.1074/jbc.M110.116004. [PubMed: 20233723]
- Simón-Manso Y, Lowenthal MS, Kilpatrick LE, Sampson ML, Telu KH, Rudnick PA, Mallard WG, Bearden DW, Schock TB, Tchekhovskoi DV, Blonder N, Yan X, Liang Y, Zheng Y, Wallace WE, Neta P, Phinney KW, Remaley AT, Stein SE, 2013. Metabolite Profiling of a NIST Standard Reference Material for Human Plasma (SRM 1950): GC-MS, LC-MS, NMR, and Clinical Laboratory Analyses, Libraries, and Web-Based Resources. *Anal Chem.* 85 (24), 11725–11731. [PubMed: 24147600]

- Takeda K, Tsukue N, Yoshida S, 2004. Endocrine-disrupting activity of chemicals in diesel exhaust and diesel exhaust particles. *Environ Sci Int J Environ Physiol Toxicol.* 11 (1), 33–45.
- Uppal K, Soltow QA, Strobel FH, Pittard WS, Gernert KM, Yu T, Jones DP, 2013. xMSanalyzer: automated pipeline for improved feature detection and downstream analysis of large-scale, non-targeted metabolomics data. *BMC Bioinformatics.* 14 (1) 10.1186/1471-2105-14-15.
- van Veldhoven K, Kiss A, Keski-Rahkonen P, Robinot N, Scalbert A, Cullinan P, Chung KF, Collins P, Sinharay R, Barratt BM, Nieuwenhuijsen M, Rodoreda AA, Carrasco-Turigas G, Vlaanderen J, Vermeulen R, Portengen L, Kyrtopoulos SA, Ponzi E, Chadeau-Hyam M, Vineis P, 2019. Impact of short-term traffic-related air pollution on the metabolome – Results from two metabolome-wide experimental studies. *Environ Int.* 123, 124–131. [PubMed: 30522001]
- Veras MM, Damaceno-Rodrigues NR, Guimarães Silva RM, Scoriza JN, Saldiva PHN, Caldini EG, Dolhnikoff M, 2009. Chronic exposure to fine particulate matter emitted by traffic affects reproductive and fetal outcomes in mice. *Environ Res.* 109 (5), 536–543. [PubMed: 19394924]
- Virág L, Szabó C, 2001. Purines inhibit poly(ADP-ribose) polymerase activation and modulate oxidant-induced cell death. *FASEB J.* 15 (1), 99–107. 10.1096/fj.00-0299com. [PubMed: 11149897]
- Vlaanderen JJ, Janssen NA, Hoek G, Keski-Rahkonen P, Barupal DK, Cassee FR, Gosens I, Strak M, Steenhof M, Lan Q, Brunekreef B, Scalbert A, Vermeulen RCH, 2017. The impact of ambient air pollution on the human blood metabolome. *Environ Res.* 156, 341–348. [PubMed: 28391173]
- Wesselink AK, Kirwa K, Hatch EE, et al. Residential proximity to major roads and fecundability in a preconception cohort. *Environ Epidemiol.* 2020;4(6):e112. doi: 10.1097/EE9.000000000000112. [PubMed: 33778352]
- Ye L, Guo J, Ge RS. Environmental Pollutants and Hydroxysteroid Dehydrogenases. In: *Vitamins & Hormones.* Vol 94. Elsevier; 2014:349–390. doi:10.1016/B978-0-12-800095-3.00013-4. [PubMed: 24388197]
- Yu T, Park Y, Johnson JM, Jones DP, 2009. apLCMS—adaptive processing of high-resolution LC/MS data. *Bioinformatics.* 25 (15), 1930–1936. 10.1093/bioinformatics/btp291. [PubMed: 19414529]
- Zhang J, Cai Z, Ma C, Xiong J, Li H, 2020. Impacts of Outdoor Air Pollution on Human Semen Quality: A Meta-Analysis and Systematic Review. *BioMed Res Int.* 2020, 1–9. 10.1155/2020/7528901.
- Zhou S.u., Xi Y, Chen Y, Zhang Z, Wu C, Yan W, Luo A, Wu T, Zhang J, Wu M, Dai J, Shen W, Zhang F, Ding W, Wang S, 2020. Ovarian Dysfunction Induced by Chronic Whole-Body PM2.5 Exposure. *Small.* 16 (33), 2000845.

A.

Metabolic Pathways	Number of metabolites in pathway	C18 Negative Mode (p<0.05)				
		NO ₂	O ₃	PM _{2.5}	Black Carbon	Mature Oocyte
Prostaglandin formation from dihomo gama-linoleic acid	3	100%				67%
Vitamin D3 (cholecalciferol) metabolism	16	44%				31%
Bile acid biosynthesis	38	32%				29%
Histidine metabolism	22	32%				
Androgen and estrogen biosynthesis and metabolism	47	26%				
Hexose phosphorylation	20		25%			
Glycine, serine, alanine and threonine metabolism	32			25%		
Vitamin A (retinol) metabolism	25			24%		
Electron transport chain	2				100%	
Urea cycle/amino group metabolism	45					20%
Ascorbate (Vitamin C) and Aldarate Metabolism	33					21%
Polyunsaturated fatty acid biosynthesis	4					50%
Caffeine metabolism	10					30%

B.

Metabolic Pathways	Number of metabolites in pathway	HILIC Positive Mode (p<0.05)				
		NO ₂	O ₃	PM _{2.5}	Black Carbon	Mature Oocyte
Vitamin D3 (cholecalciferol) metabolism	10	70%		40%		50%
C21-steroid hormone biosynthesis and metabolism	53	38%			21%	34%
Androgen and estrogen biosynthesis and metabolism	24	46%				33%
Vitamin A (retinol) metabolism	22	41%		36%		36%
Carnitine shuttle	35	34%				43%
Beta-Alanine metabolism	13	46%				
Bile acid biosynthesis	33	33%	27%		18%	27%
Ascorbate (Vitamin C) and Aldarate Metabolism	11		36%			
Prostaglandin formation from arachidonate	37		22%			
Phosphatidylinositol phosphate metabolism	25		24%			
Hyaluronan Metabolism	4		50%			
Biopterin metabolism	16			44%		
De novo fatty acid biosynthesis	20			35%		
Vitamin E metabolism	26			31%		
Vitamin B2 (riboflavin) metabolism	3			67%		
C5-Branched dibasic acid metabolism	2				100%	
Prostaglandin formation from dihomo gama-linoleic acid	6				50%	
Arachidonic acid metabolism	30				23%	
Tyrosine metabolism	110					24%
Propanoate metabolism	3					67%
Squalene and cholesterol biosynthesis	15					33%

*Percentages in the cells represent the proportion of overlapping metabolites

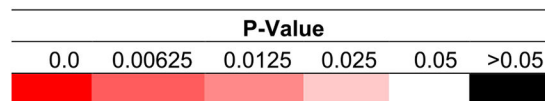


Fig. 1. Metabolic pathways associated with air pollution and mature oocytes in the C18 column with negative ESI (Panel A) and HILIC column with positive ESI (Panel B) platforms.

*Percentages in the cells represent the proportion of overlapping metabolites.

Significant metabolic features associated with NO₂, O₃, PM_{2.5}, and BC exposure and mature oocyte among 125 women in the EARTH study.

Table 1

	C18 Negative (N = 14394)			HILIC Positive (N = 17161)		
	Raw < 0.05	Raw < 0.005	Raw < 0.0005	FDR < 0.20	FDR < 0.05	FDR < 0.05
NO ₂	1317	128	21	8	5	19
O ₃	521	59	9	0	0	13
PM _{2.5}	1166	90	12	0	0	0
BC	599	55	6	0	0	0
MII Oocytes	897	111	15	3	1	0

(NO₂ = Nitrogen Dioxide; O₃ = Ozone; PM_{2.5} = Fine Particulate Matter; BC = Black Carbon; MII oocytes = total number of Mature oocytes).

Table 2

Chemical identity of the annotated metabolites in the follicular fluid significantly associated with at least one air pollutant and number of mature oocytes.

<i>m/z</i>	<i>RT</i> ¹	<i>m/z</i> error (ppm)	Validated Metabolite	Adduct Form	Associated Pollutant	% Change (95 % CI) per SD Increase in Pollutant	% Change (95 % CI) per Mature Oocyte Retrieved	Column	Level of Evidence
130.0873	23	3.6	L-ISOLEUCINE LEUCINE NORLEUCINE	M-H	Black Carbon	-14 (-24, -3)	-3 (-6, -1)	C18 neg	1
377.0863	22.2	1.9	D-LACTOSE SUCROSE MELIBIOSE MALTOSE D-(+)-CELLOBIOSSE PALATINOSE	M + Cl	NO2	51 (14, 102)	-7 (-13, -2)	C18 neg	1
391.287	170.9	5.5	DEOXYCHOLATE	M-H	PM2.5	-18 (-30, -4)	-5 (-8, -2)	C18 neg	1
195.0875	30.4	-3.5	CAFFEINE	M + H	NO2	47 (15, 88)	5 (0, 11)	HILIC pos	1
131.0825	24	3.2	D-ORNITHINE L-ORNITHINE	M-H	Black Carbon PM2.5	-13 (-23, -2) -13 (-22, -2)	-3 (-5, -1)	C18 neg	2
135.0302	23	-3.8	HYPOXANTHINE	M-H	NO2	11 (1, 22)	-3 (-5, -1)	C18 neg	2
118.0863	60.7	-4.1	L-VALINE L-NORVALINE 5-AMINOPENTANOATE	M + H	PM2.5	-10 (-17, -2)	-3 (-4, -1)	HILIC pos	2
176.103	93.5	-2.8	CITRULLINE	M + H	PM2.5	-14 (-22, -5)	-3 (-5, -1)	HILIC pos	2

¹ Retention time was measured in seconds.