

Non-Targeted Assessment of Environmental Contaminants and their Correlations with Semen Health Factors in New York City

Trevor A. Johnson¹, Sarah Adelman¹, Bobby B. Najari², Joshua F. Robinson³, Linda G. Kahn^{1*}
and Dimitri P. Abrahamsson^{1*}

¹New York University, Grossman School of Medicine, Department of Pediatrics

²New York University, Grossman School of Medicine, Department of Urology

³University of California San Francisco, Department of Obstetrics, Gynecology &
Reproductive Sciences

*These authors contributed equally to the manuscript

Abstract

Characterizing chemical composition of semen can provide valuable insights into the exposome and environmental factors that directly affect seminal and overall health. In this study, we compared molecular profiles of 45 donated semen samples from general population New York City participants and examined the correlation between the chemical profiles in semen vs. fertility parameters, i.e., sperm count, sperm morphology, sperm motility and semen volume. Samples were prepared using a protein precipitation procedure and analyzed using liquid chromatography (LC) and high-resolution mass spectrometry (HRMS). Non-targeted analysis (NTA) revealed eighteen chemicals not previously reported in human exposome studies, with 3-hydroxyoctanedioic acid, a cosmetic additive, emerging as a plausible candidate found to be at higher levels in cases vs. controls ($p < 0.01$) and associated with adverse sperm motility and morphology.

This study aims to be the first of its kind to utilize NTA to understand the association of contaminants of emerging concern (CECs) along with a full chemical profile to find trends separating poor and normal semen health parameters from each other chemically. Our results suggest that the collective effects of many CECs could adversely affect semen quality.

1 Introduction

Male infertility

Infertility is defined as the failure to establish clinical pregnancy after 12 or more months of regular, unprotected intercourse. Worldwide it is estimated to impact up to 12% of couples within reproductive age, with 50% of overall cases due to male-specific infertility [1]. Critically, male-specific infertility is an indicator of poor overall health, with increased rates of cancer [2,3], heart disease [4,5], diabetes [6] and early mortality [4,7,8] all corresponding with a decline in male reproductive health [9,10]. Over the nearly thirty-year period between 1990 and 2019 the rate of male infertility is estimated to have increased by 75-80% [11,12,13]. Trends are most drastic for younger males. Those under 30 years of age have experienced a 15% greater impact than those over 30 years of age in the same timeframe [14,15]. Though fewer than 10% of men with fertility issues in the United States seek regular services to solve them, those who do can spend up to 20% of their annual income on treatments and expenses [16]. There is, therefore, a significant economic impact from male infertility, not only in terms of healthcare costs associated with coinciding medical issues, but also fertility treatment.

The semen exposome and environmental contributors

Environmental exposures to anthropogenic pollutants are suspected to contribute to the historical decline in male reproductive health and semen quality [17,18,19]. Industrial, high production pollutants, such as chlorophenols, nitrophenols, vinyl chloride, epichlorohydrin, phthalates,

acetaldehyde, and other antiandrogens have all produced animal and human fertility decline in controlled *In Vitro* studies by disrupting enzyme function, changing androgen-targeting tissues and altering spermatogenesis and sperm morphology (physical shape and characteristics) [17,19]. Heavy metals and pesticides have also been shown to negatively impact the mechanism and effect of male reproductive organ processes through *In Vivo* animal and human epidemiological studies [17,18,19]. There is growing understanding of which exogenous compounds are present in human semen [20]. Chemicals found range from clear anthropomorphic sources, such as pesticides, phthalates and pharmaceutical products, to more ambiguous and naturally occurring sources, such as purines, furans, benzaldehydes and organic acids [21]. Recent studies have aimed to examine associations between specific chemical classes and semen health. For example, halogenated compounds, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and Polyfluoroalkyl Substances (PFAS) have shown limited association with poor semen health outcomes (sperm concentration, sperm motility, sperm morphology, seminal volume and total sperm count) [22,23]. Of particular interest are contaminants of emerging concern (CEC), which are becoming more prevalent in the modern environment [24,25,26,27]. CECs include pesticides, such as atrazine and diamino chlorotriazine, persistent organic pollutants (POPs) like chlorophenols, industrial by-products such as benzotriazoles and dioxins, flame retardants/suppressors and surfactants such as polybrominated diphenyl ether and perfluorooctanoic acid (PFOA). PFAS, phthalates, and heavy metals have been the three most analyzed groups in human blood, semen and urine in recent years using targeted methods [28,29]. Many of the methods assessing exogenous contributions to poor semen focus on the many environmental factors that produce them. Smoking, for example, introduces many toxins directly into the lungs through primary and secondary exposure [30]. Industrial products, including bisphenols, phthalates, heavy metals, nitrophenols and acetaldehydes can be introduced through

water, air and in contaminated food [31,32,33]. Non-targeted analysis (NTA) approaches designed to analyze a wide range of known and potentially new chemical sources are seeing increased use in medical research. Previously unknown sources of disease have been found, such as liquid crystal monomers found in dust and soil [34], and benzopyran-based pesticides and phenolic industrial waste in blood and prenatal samples [35,36]. The impact of individual CECs and distinct classes of CECs have been well studied [24,25,26,27]; however, the combined environmental presence, impact and treatment of many different classes of CEC together in the environment is less known and would utilize the chemical coverage offered by NTA using LC-HRMS [24,37]. Furthering this knowledge could improve the understanding of male reproductive and overall health more than the study of individual chemicals and classes [31,38,39,40].

Exposomic profiling using non-targeted MS-based approaches

NTA, by design, does not limit itself to individual chemicals or chemical classes. It can screen thousands of chemicals in case and control patients to look for new features of interest [40,41,42,43]. Identification can often be challenging with complex matrices. High-resolution mass spectrometry (HRMS) separates based on very small differences in mass, which is crucial to the identification and intensity of individual ions in NTA [43]. Examples of HRMS-based chemical-disease relationship and/or biomarker studies include the relationship between di-isononyl phthalate metabolites and liver disease [40], and the identification of hundreds of pharmaceutical biomarkers in human urine and saliva [44,45].

HRMS identification and validation should include consistent detection of compounds across replicates and should attempt to utilize known standards for confirmation [43]. Databases such as the United States Environmental Protection Agency (USEPA) CompTox Chemicals Dashboard (henceforth referred to as the “CompTox Dashboard”), a regularly updated database containing

diverse information (e.g., physicochemical properties, environmental fate and transport, exposure, usage, in vivo and in vitro toxicity) on over 1,200,000 known chemicals [46], the Human Blood Exposome database [47], and PubChem through the National Institute of Science and Technology (NIST) [48] are constantly expanding. In addition, tools such as the Metaboanalyst® [49] exposure enrichment tool, can be used. The exposure enrichment tool accepts a list of compound names and concentrations, analyzing them against 15 libraries containing ≈13,000 biologically meaningful metabolite sets collected primarily from human studies including >1500 chemical classes, providing tentative insight into the source of metabolites in biological samples. These databases and tools grant new opportunities to better define chemical exposures link them to outcomes.

In this study, we plan to use NTA to profile chemicals in semen of young adult men in New York City. We aim to discover compounds that have been overlooked using conventional targeted methods. Our analysis will measure infertility parameters, i.e., sperm concentration, sperm motility, sperm morphology and semen volume, providing associations between the chemical profiles and sperm quality. Finally, using the exposure enrichment tool, we will use the chemicals associated with these trends to highlight exposure pathways that are prevalent in low quality semen compared to healthy controls.

2. Methods

Individual steps of the experimental and data processing aspects of the study are presented in Figure 1.

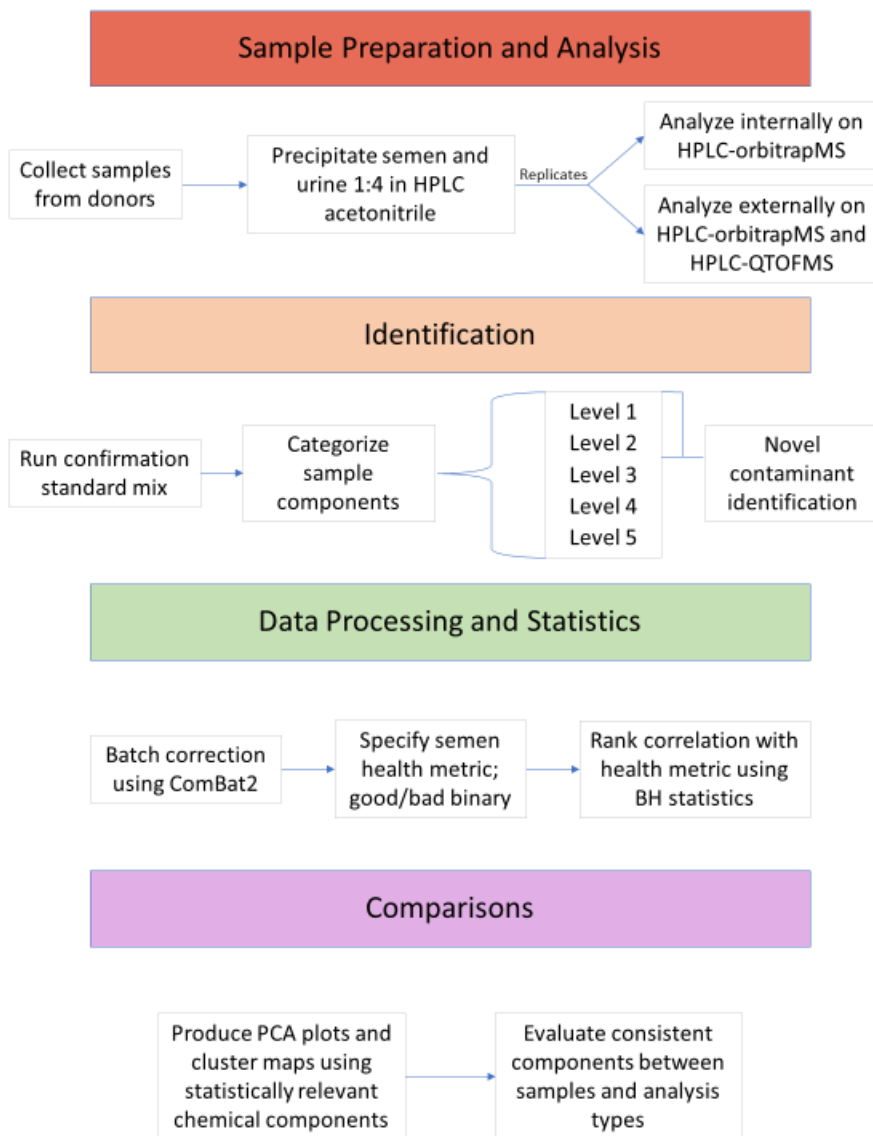


Figure 1: Workflow diagram for experimental and data processing steps.

2.1 Participant Information, Sample Collection and Storage

Between September 2022 and February 2023, 45 healthy men within the eligible range of 18-45 years of age from the New York City area enrolled in the study. All participants were informed about the study in various ways, including social media posts, physical flyers, and an internal system called iConnect. Each participant reported no diagnosed medical condition or use of any

medication or supplement associated with poor semen quality. After providing informed consent, each filled out a baseline questionnaire before attending a single study visit. Up to 24 hours prior to their study visit, participants completed a 24-hour diet recall survey [50]. On the day of the study visit, they provided a semen sample at a local andrology lab (Repro Lab) following 2-7 days of ejaculatory abstinence, then walked to the NYU Environmental Pediatrics research clinic where they had anthropometric measurements taken and provided a urine sample. Participants had a mean age of 28.9 years (range: 23-38) and median body mass index of 24.2 kg/m² (interquartile range [IQR]: 23.0-26.3).

Semen samples were analyzed following liquefaction at room temperature in accordance with World Health Organization 1999 and 2010 reference recommendations [51], and standard semen parameters were reported. The remaining samples were aliquoted into 1.8 mL polypropylene tubes and frozen at -80 °C before being transported to NYU Langone Health for analysis.

2.2 Semen Health Parameters

Table S4 in the Supporting Information provides details on semen health parameters collected from individual donors. Thresholds for dichotomized high/low values were based on the values outlined in the WHO 1999 and 2010 reference values [51,52]. Each of the semen quality parameters were given binary values (i.e. a value of 0 if above the WHO reference level, a value of 1 if below the reference level). Sperm morphology, or the size and shape of sperm when viewed with a microscope, was assessed to give samples a percent normal morphology. For the purposes of this study 30% normal sperm morphology was used as the upper limit for low morphology. The WHO criterion for low motility was an upper limit of 50% motile, and the threshold for low concentration/count was 20×10⁶/mL. These values were all interpreted to have clinical relevance for male fertility [53]. An upper threshold of 2 mL was used for low semen volume.

2.3 Standard Makeup and Analysis

An external standard solution for compound retention time and mass confirmation was made from three mixes of selected compounds of potential interest. These compounds range from known pesticides to food additives, over-the-counter drugs, vehicle fluids, and cleaning components. The full list of compounds and their identification information is included in Table S1 in the Supporting Information. Dilutions were made so that the concentration of each analyte was equivalent to 100 ppb (100 µg/L or 1/10000000 v/v) in acetonitrile.

2.4 Protein precipitation

Frozen 1.8 mL aliquots of semen were removed from -80 °C and allowed to thaw for a minimum of 1 hour before extraction. All volumetric pipetting was done with a 200 µL adjustable Reference2 pipette (Eppendorf, Enfield, CT) with 200 µL tips (Finntip, Thermo Fisher Scientific, Waltham, MA). Figure 2 shows a schematic of the sample preparation and analysis procedure. 100 µL of sample was pipetted into a 1.7 mL polypropylene microcentrifuge tube (Corning, Phoenix, AZ), followed by 400 µL of HPLC grade acetonitrile (Fisher Scientific, Hampton, NH). The mixture was vortexed for 20 s at 1500 rpm using a 100-240V lab vortex mixer (Thermo Fisher Scientific, Waltham, MA) and allowed to stand for 20 min. Samples were then centrifuged for 10 min at 3000 rpm using a miniSpin 5452 centrifuge (Eppendorf, Enfield, CT). A glass Pasteur pipette was then used to transfer the supernatant to a 3 mL syringe with a 0.2 µm pore size filter (Monoject, Cardinal Health, Dublin, OH), which was then used to dispense the final precipitated and filtered sample into a 300 µL polyspring amber glass insert vial (Thermo Fisher Scientific, Waltham, MA). One method blank of 400 µL acetonitrile and 100 µL HPLC grade water (Fisher Scientific, Hampton, NH) was prepared identically to samples for every eleven real samples (five method blanks for each set of replicates).

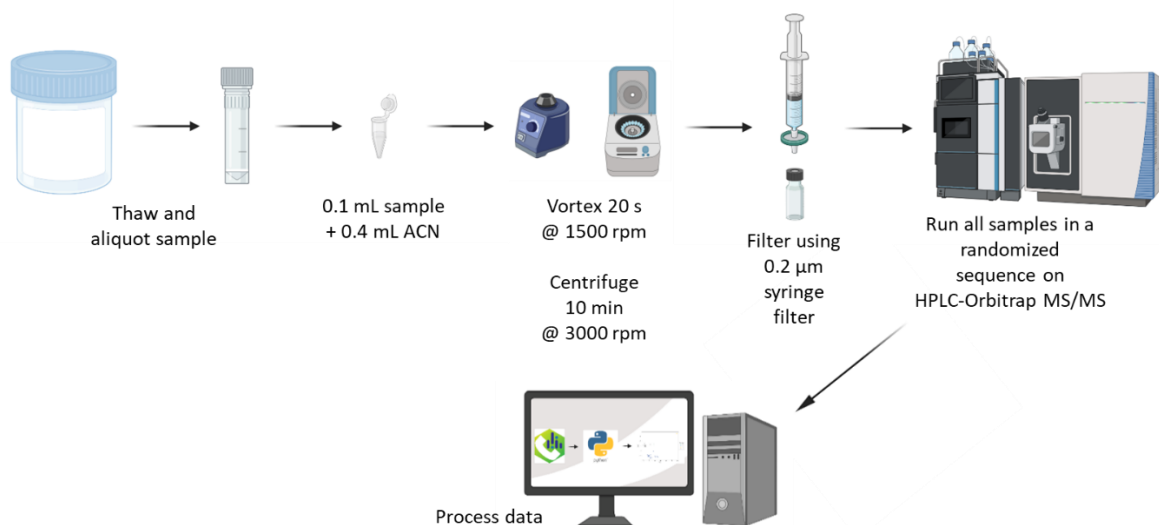


Figure 2. Schematic of sample handling, protein precipitation and analysis, followed by processing using MS Dial software and Python-based data tools.

2.5 Non-targeted LC-MS/MS Analysis

Analysis was performed using a Vanquish Horizon UHPLC System coupled to an Exploris 240 Orbitrap (both Thermo Fisher Scientific, Waltham, MA). Details of mobile phases, flow information and mass spectral parameters can be found in Table S2 of the Supporting Information. Briefly, the aqueous phase (solvent A) was 99.9% HPLC water with 0.1% HPLC methanol, while the organic phase (solvent B) was 90% HPLC methanol with 10% HPLC water. LC solvents were all obtained from Fisher Scientific (Hampton, NH). Both solvent mixtures had 5 mM ammonium acetate added. The analytical column used was a 2.1×100 mm, 1.8 µm pore size Eclipse Plus C18 column. Flow was set to a constant 0.150 mL/min, with the following solvent flow gradient: start at 10% solvent B for 1 min, ramp to 100% solvent B for 14 min, hold at 100% solvent B for 5 min, re-equilibrate at 10% solvent B for 3 min. Mass voltage for positive ionization mode was set to 3500 V and scanned for 105-1050 m/z with a resolution of 60 000. Negative ionization mode was set to 2500 V with identical m/z and resolution settings. Five quality control (QC) samples were run within the sequence using

400 μ L of the 100 ppb standard mixture in acetonitrile and 100 μ L HPLC grade water. This QC mixture consisted of Mixture 1 from Table S1 and was used to ensure consistent mass and retention time values for analytes. QC, method blank and real samples were run together in a randomized order, two replicates per ionization mode (positive/negative).

2.6 Raw File Processing and Identification

Raw files obtained from the MS system were processed using MS-Dial software [53]. Details of parameters used are included in Table S3 in the Supporting Information.

Mass spectral libraries MSMS_Public_EXP_Pos_VS17 (positive ionization) and MSMS_Public_EXP_NEG_VS17 (negative ionization) were used for mass spectral matches, with additional matches done through PubChem FTP. MS/MS matches of 70/100 and higher were categorized as Level 2 identified, while MS matches of 70/100 and higher were categorized as Level 3. Molecular formula matches based on mass were categorized as Level 4, with unknowns generating an accurate mass measurement being categorized as Level 5. Literature research was done on Level 1 and Level 2 chemicals, as well as those that produced notable molecular formulae. Level 2 spectral matches that did not meet visual inspection comparison were downgraded to level 3. A shortlist of level 1 and level 2 compounds that did not appear in Blood Exposome Database [47] searches was made, noting them as potentially novel or understudied. Identification standards (Table S1) were used to determine the presence of a list of suspect compounds within samples. Compounds that consistently matched retention time within 0.3 min retention time and 5 ppm mass were categorized as Level 1 identified based on the guidelines by Sumner et al, 2007 [54]. A full list of level 1 identified compounds is included in Spreadsheet S7 of the Supporting Information.

2.7 Data Processing

The first step in preparing the data was to eliminate peaks that were suspected to be instrument noise. A peak area of 10000 AU was set as the minimum with all peaks below set to zero. We then applied a normal distribution to data points using calculated median and standard deviation values from experimental data. The model then produced random values between the minimum experimental value and the absolute minimum (zero) following the shape of the distribution. Peaks with significant intensity in blanks (within 100000 AMU area of value in sample) were removed from sample data tables. The initial processing steps dictate the minimum measured value, based on a set cut-off point in the chromatograms. This point, providing a safe margin from the baseline, is set in NTA studies due to the unknown method detection limit. The code for the imputation is available as Supporting Information on GitHub (https://github.com/johnsont114/UrineSemen_UsedinProcessing) as “imputation.py”.

Batch correction was performed using a Combat2 algorithm. Batches of samples were separated into groups of 12 (11 samples and 1 blank) to form preparation batches, and groups of 50 (samples run in a single day) to form instrument analysis batches. QC samples containing mixture 1 from Table S1 were run along with each batch (inter-preparation-batch and inter-day) to support that batch correction statistically removed variation observed between batches and that variation was coming from samples, not batch numbers. Example principal component analysis (PCA) plots of these corrections are displayed in Figure S1 in the Supporting Information.

2.8 Statistics and Interpretation

We used a regression model to examine the relationship between each compound and each semen quality parameter. To ensure that our results were not skewed by the large number of comparisons we were making, we used a technique called the Benjamini-Hochberg (BH) false

discovery rate [55]. This method helps us control the chance of getting false positives in results by setting a threshold for significance.

We then used Principal Component Analysis (PCA) to further analyze the compounds that showed the greatest significance in our t-tests. The compounds that were considered highly significant were those that met the criteria of Equation 1. In this equation, 'l' represents the rank of the p-value, 'm' is the total number of values, and 'Q' is the false discovery rate, which we tested at 0.05 and 0.10. These leave a 5% and 10% chance of getting a false positive in our results, respectively. The full processed datasets for positive and negative ionization are included as supplementary spreadsheets on GitHub (https://github.com/johnsont114/NTA-Semen-NYC_ProcessedData).

Equation 1
$$\frac{l}{m}Q - pvalue \leq |0.05|$$

To obtain chemical exposure enrichment information a list of compounds with $p < 0.05$ association with each semen health parameter were input into Metaboanalyst® 6.0 exposure enrichment analysis [49]. Metaboanalyst® functions using compound name, so a minimum of level 3 identification was required for input. All three levels (level 1, level 2 and level 3) were used to produce enrichment diagrams.

3 Results

3.1 Morphological Characteristics of Participants

Table 1 is a summary of the clinical statistics collected for each of the study participants. Table S4 in the Supporting Information details the experimentally measured morphological characteristics of study participants.

Table 1. A summary of the clinical statistics and semen health parameters of the study participants.

Concentration ($\times 10^6/\text{mL}$)	Motility (%)	Morphology (% normal)	Volume (mL)
---	--------------	-----------------------	-------------

Mean	72.1	44.5	58.1	2.4
Median	56.5	45	60.5	2.5
Max	208	75	81	5
Min	4	16	2	0.3
Range	204	59	79	4.7

	Low Conc	Low Morph	Low Mot	Low Vol
Low Count	6 (13%)	11 (24%)	6 (13%)	14 (31%)

45 participants took part. 6 participant semen samples had what was considered low sperm concentration, 11 had a low sperm motility, 6 had a low sperm morphology, and 14 had low semen volume. Two samples (sample #11 and sample #32) were identified before analysis to be collection outliers and were removed from the study.

3.2 Non-Targeted Overview and Compound Associations with Semen Health

Displayed in Table 2 are the compounds identified at different levels of confidence throughout the study based on the identification guidelines by Sumner et al, 2007 [54]. Between all combined semen samples, 48595 chemical features were detected. 7348 were tentatively identified with MS (minimum level 3), 363 with MS/MS (minimum level 2), and 33 had their identities supported with standards (level 1). Table 2 also displays the number of features at levels 1, 2 and 3 that were associated in some way (positively or negatively) with each semen health parameter with a t-test p-value less than 0.05.

Table 2. Number of compounds detected at each level of identification confidence and the number associated with low sperm morphology, motility, and concentration.

Identification confidence	Cumulative, agnostic to association	Associated with concentration (+/-)	Associated with motility (+/-)	Associated with morphology (+/-)	Associated with volume (+/-)
1	33	0	2	2	1
2	363	29	77	18	29
3	7348	272	642	274	270

All 48595 2014 4803 1807 1818

Eighteen previously unreported exposomic chemicals were identified at level 2 confidence using MS/MS spectra and libraries (4 in negative mode, 14 in positive mode). Novelty was determined by comparing the compounds' identification information, formula and SMILES with the existing Blood Exposome Database [47], which, since no semen-specific database exists, we deemed the most relevant database to use in this study. Manual searches were also done in Google Scholar for research specific to each chemical. Four of the eighteen unreported chemicals were associated with semen health parameters ($p < 0.05$) and are included in Table 3. Comparison of experimental and literature mass spectra only yielded 3-hydroxyoctanedioic acid as a plausible candidate. Box plots comparing chromatographic peak abundances between cases and controls are shown in Figure 3. The two associations for 3-hydroxyoctanedioic acid showed significant differences ($p < 0.01$) between cases and controls. 3-hydroxyoctanedioic acid was associated with low motility (Figure 3A) and low morphology (Figure 3B). A full list with details of the eighteen potentially novel compounds is included in Spreadsheet S6 in the Supporting Information. Visual comparisons of experimental and literature spectra are included in Table S5.

Table 3. Compound information for potentially novel compounds found to have relative association with semen quality.

DTXSID	IUPAC Name	Associated Health Factor	Association (+/-) ^b	Literature Spectrum Match
DTXSID20624863	3-Hydroxyoctanedioic acid	Motility, Morphology	-	Y
N/A ^a	5-[5-hydroxy-3-(hydroxymethyl)pentyl]-8a-(hydroxymethyl)-5,6-dimethyl-3,4,4a,6,7,8-hexahydronaphthalene-1-carboxylic acid	Concentration	-	N
N/A ^a	6,10a-dihydroxy-4-(hydroxymethyl)-4,7,11b-trimethyl-1,2,3,4a,5,6,6a,7,11,11a-decahydronaphtho[2,1-f][1]benzofuran-9-one	Concentration	-	N
DTXSID40904194	4'-ethenyl-2'-hydroxy-1,4',4a-trimethyl-5-oxospiro[2,3,4,7,8,8a-hexahydronaphthalene-6,1'-cyclopentane]-1-carboxylic acid	Volume	+	N

^a Those that did not present a DTXSID were those not in the CompTox Dashboard. They were, however, included in the PubChem database without DTXSID. ^b Association: These are split into one of two

categories; (+) if an increased amount of the chemical was related to an improvement in the health parameter of interest, and (-) if an increased amount of the chemical was related to a decline in the health parameter of interest.

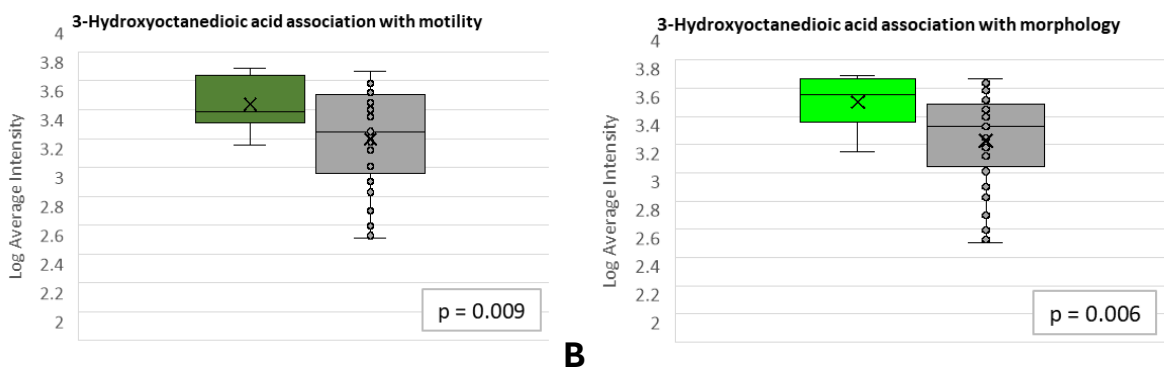


Figure 3. Box plots of semen health factors associated with 3-hydroxyoctanedioic acid. p-values displayed are those comparing the low and normal health parameters of (A) motility and (B) morphology. Green boxes (left side of each plot) indicate cases with low health parameters, gray boxes (right) indicate controls.

Positive and negative MS ionization modes were treated separately for the purposes of trend elucidation. Figure 4 presents data on chemicals with $p \leq 0.05$ for sperm concentration. $\approx 4\%$ of all features within the samples had a p -value ≤ 0.05 and were included in the dataset used. Peak tables used to produce PCA plots are included in GitHub (https://github.com/johnsont114/NTA-Semen-NYC_ProcessedData). It should be noted that PCA is a metric of variance and not of quantitation. For this study PCA was used to reduce the dimensionality of the dataset and examine how different semen control samples were compared to case samples in terms of their aligned chemical composition.

We were able to find trends when observing chemical features for each of the health parameters measured. Figures 4 and 6 show the separation observed for low concentration and low volume, while Figure S2 and Figure S3 in the Supporting information include the corresponding plots for low

motility and low morphology. The positive ionization of low concentration (Figure 4A) showed trends away, but not clear separation from healthy concentration samples. This is reflected in the PC1 p-value of 0.063. In negative ionization (Figure 4B), however, there was clearer separation in PC1 with a low p-value of 7.23×10^{-7} . The separation between the two groups (low concentration and normal concentration) in negative ionization mode are also illustrated in a clustermap of the features Figure 5. The low concentration samples (species) are nearly entirely grouped together, indicating that their chemical profiles are very similar compared to controls.

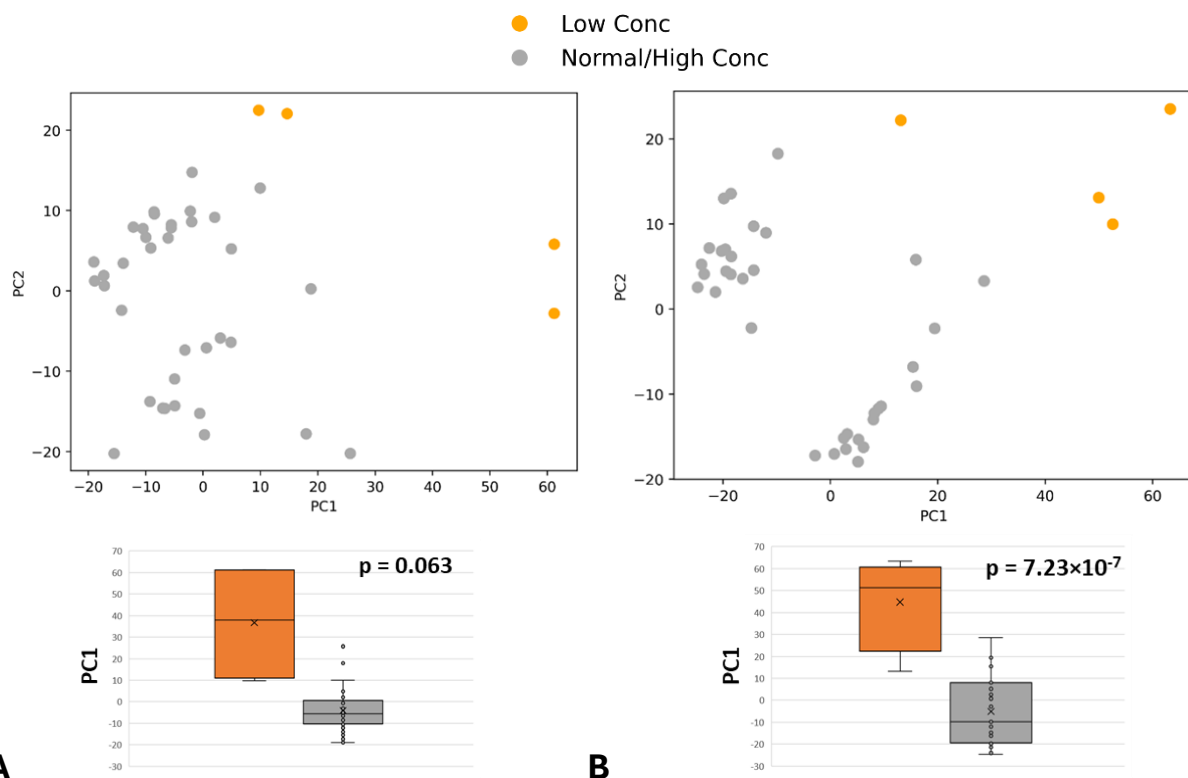


Figure 4. PCA plot for semen samples including only the chemicals with the lowest p-values correlating with either low or normal/high sperm concentration in semen. (A) Positive ionization and (B) negative ionization are shown. P-values for the first principal component are included along with a box plot of the principal component for each.

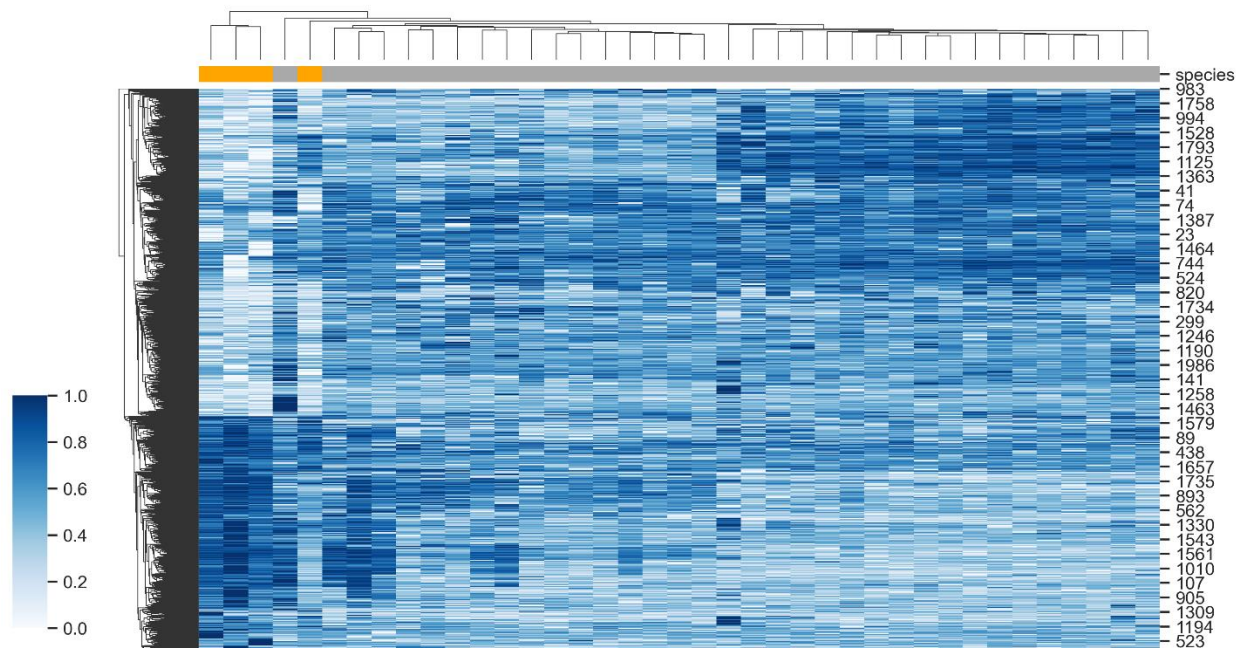
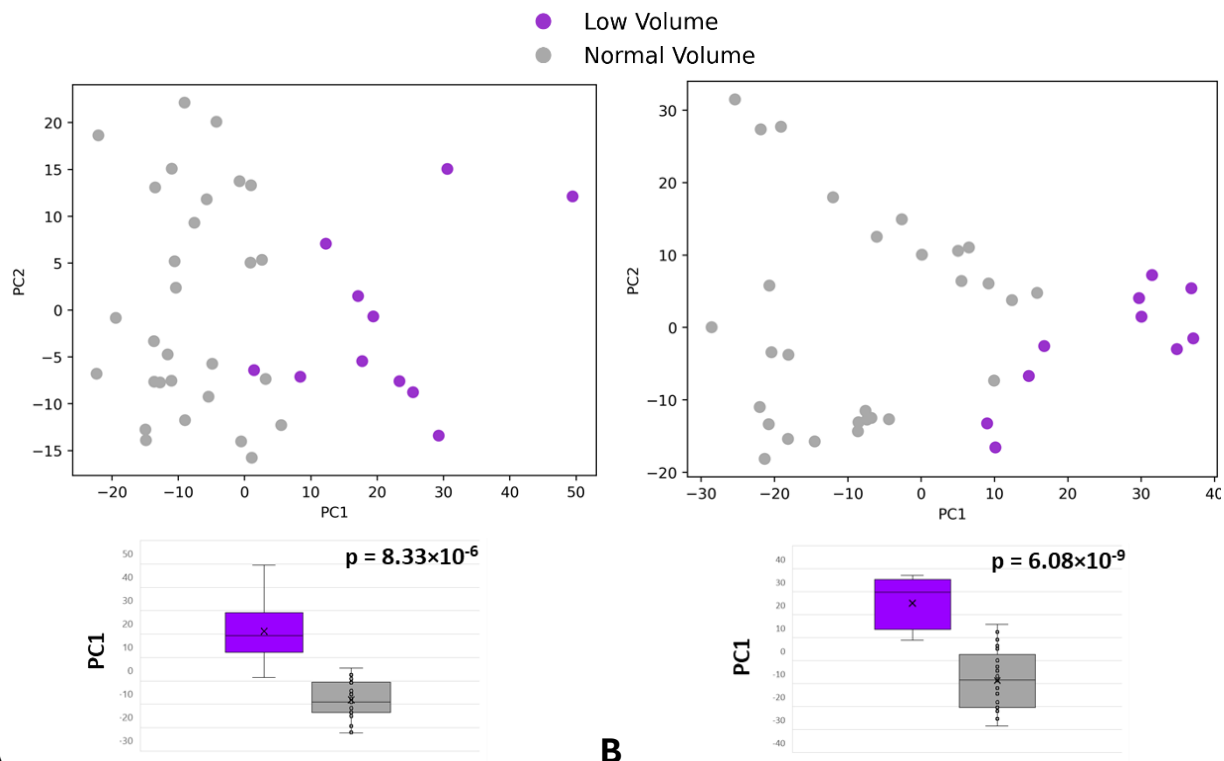


Figure 5. Clustermap of the features in the low concentration dataset. Flags for low concentration samples (species) are indicated in orange, with normal concentration indicated in gray.

Figures 6A and 6B show the PCA information for low volume datasets (positive and negative, respectively). Low volume had the highest number of cases, with 31% of samples being categorized with low volume. Both ionization modes show significant variance between cases and controls in PC1, as shown by the low p-value calculated for each. Figure 7 is the clustermap of the positive ionization features for low volume. Most low volume samples (species) are clustering, and a p-value was calculated for the plot at well below 0.001. All clustermaps for semen health parameters are in Figures S4-S7 in the Supporting Information.

320



321

A

B

322 Figure 6. PCA plots for semen samples including only the chemicals with the lowest p-values correlating
 323 with either low or normal semen ejaculate volume. (A) Positive ionization and (B) negative ionization are
 324 shown. P-values for the first principal component are included along with a box plot of the principal
 325 component for each.

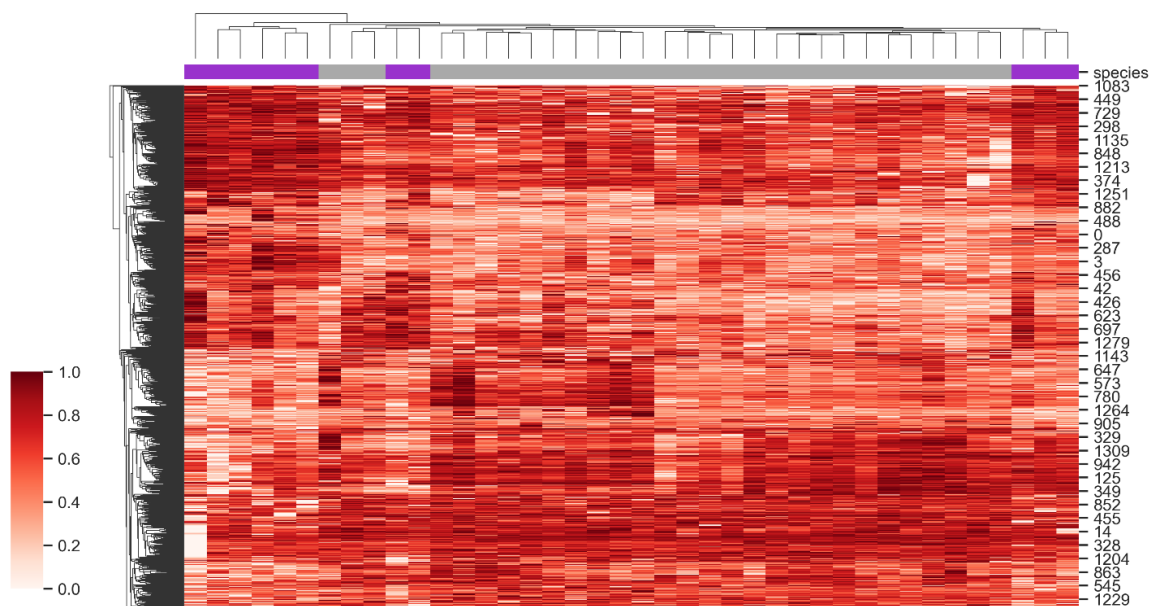


Figure 7. Clustermap of the positive ionization features in the low volume dataset. Flags for low volume samples (species) are indicated in purple, with normal volume indicated in gray.

3.3 Exposure Pathway Analysis

Seen in Table 4 is the Metaboanalyst® 6.0 exposure enrichment data produced when the level 1, level 2 and level 3 identified compounds associated with semen health parameters were input. No internal process was used to confirm or validate the figures or pathways, and exposure enrichment does not consider concentration or intensity of chemicals for its calculations. Therefore, the results should be considered tentative to provide insight to the behaviour of chemicals and metabolites identified in this study. The pathways were required to have a raw p-value of ≤ 0.001 and a false discovery rate (FDR) of ≤ 0.05 . Low concentration, motility and volume all indicated that the most significant pathway associated was traffic-related air pollution exposure, with the only consumption-related pathway being marjoram consumption in low motility.

Table 4. Metabolite enrichment overviews of level 3 and higher identified chemicals with a $p \leq 0.05$ relationship with each low health parameter. Figures were generated using identities from both positive and negative ionization modes.

Exposure Pathway	Total ^a	Hits ^b	Raw p ^c	FDR ^d
Concentration				
Traffic-Related Air Pollution Exposure	24	5	0.000303	0.0188
Motility				
Traffic-Related Air Pollution Exposure	24	8	2.34E-05	0.00145
Marjoram Consumption	4	3	0.000944	0.0293
Morphology ^e				
Volume				
Traffic-Related Air Pollution Exposure	24	6	0.000573	0.0355

^a Total: the total number of metabolites in the specific pathway in the database. ^b Hits: number of metabolites/chemicals contributing to the exposure pathway. ^c Raw p: the unadjusted p-value obtained from the enrichment analysis; values above 0.001 were discarded. ^d FDR: The false discovery rate calculated for the pathway; values above 0.05 were discarded. ^e Morphology did not have any pathways that met the criteria for raw p and FDR.

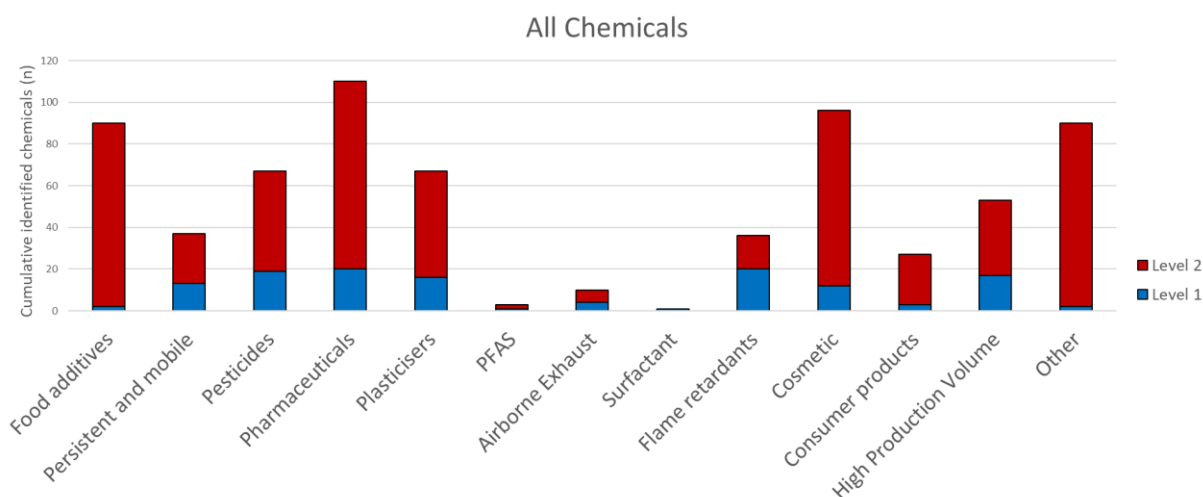
4 Discussion

A major aim of this study was to find associations between semen health outcomes and chemical trends through NTA. HRMS can make very precise chemical mass measurements, providing a distinct advantage for compound identification and comparative measurements of instrument response between the same chemical in different samples. Our results indicate potential chemical associations between environmental pollutants and semen health. Furthermore, the identification of multiple new chemical associations with health parameters suggests that the chemical drivers of poor male fertility are complex and can come from many sources.

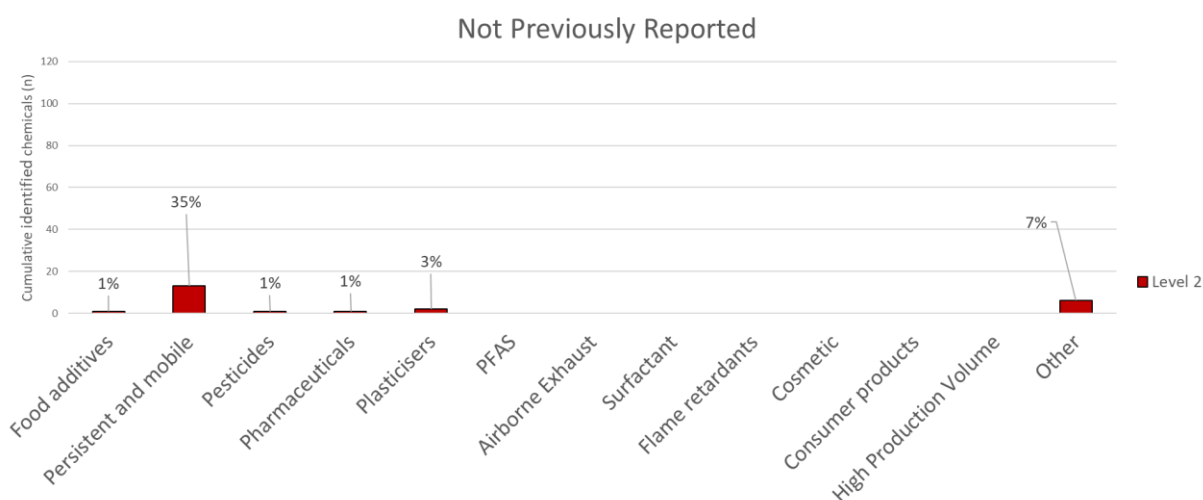
Level 1 and level 2 identified compounds fell into 32 classes of compound, detailed in Figure S8 in the Supporting Information, were obtained using ClassyFire [56]. Fatty acyls and carboxylic acids were the most represented classes of compound, with benzenes/benzene derivatives and

organooxygens as the joint third most represented. Many of the benzenes and benzene derivatives are notable since they are common in exogenous processes, such as petroleum use, plastics production and in pesticide production. Previous studies have put an emphasis on identification of certain chemicals that come from specific pollutant sources. Sources most often include food additives, pesticides [57,58], pharmaceutical drugs, plasticizers [59], PFAS [60], surfactants, flame retardants and cosmetics. Even semen analysis studies marketed as non-targeted tended to focus important findings to chemicals within these classes [21]. Figure 8 includes level 1 and level 2 identified compounds and processes that commonly produce CECs. The chemicals included in Figure 8A are all chemicals identified in the study, not necessarily those new to the exposome or associated with semen health outcomes. Figure 8B includes only compounds that were identified as new to the human exposome when compared to the Blood Exposome Database [47]. The common categories in literature are included as sources of interest, along with a few additions. Persistent and mobile compounds are compounds that tend to be prevalent consistently in the environment, whether this is because of their fast transport and mobility or difficulty in breaking them down. Airborne exhaust was added based on the results observed in Table 4 and the likelihood of traffic-related air pollution exposure in samples. Consumer products and high production volume chemicals are of interest based on the number of available chemicals in each category. They have also been included in past non-targeted exposomic studies [61]. The category “Other” encompasses chemicals which have unknown uses or do not fit into the categories previously listed. Unknown uses can stem from proprietary materials in industry, pesticide or consumer product production [43,62]. Sources were queried using the list search function of the CompTox database [46]. Since there is no chemical or metabolomic database specific to semen, the Blood Exposome Database [47] was used to determine if a chemical had been previously reported in human exposure studies. The chemical sources and the cumulative number of

chemicals have a similar profile to other chemical suspect screening papers, such as Wang et al, 2021 [61], who had nine categories of compounds identified using the 2021 version of the CompTox Database list search.



A



B

Figure 8. Chemical use information for level 1 and level 2 identified chemicals. (A) All annotated chemicals and (B) chemicals not previously reported in human exposure studies determined by those chemicals that did not appear in Blood Exposome database searches [47]. Chemical use information was collected from chemical batch lists on the EPA CompTox Dashboard [46]. Percentages of each category that were not previously reported are indicated.

Like most biological processes, fertility is intricate and requires many considerations. The decline in global fertility rates in recent years cannot entirely be placed on biological factors [11,12,13]. Environmental factors' impact on fertility cannot be underestimated. A notable relationship has previously been shown between poor semen health and fuel-burning air pollution, with PM_{2.5}, PM₁₀, SO₂, NO₂ and PAH having the most consistent associations [63,64,65]. The pathway enrichment results in our study indicated traffic-related air pollution was by far the most impactful exposure pathway. This could suggest one of three scenarios: that the metabolites detected and identified in this study act as biomarkers for pollutants such as PM_{2.5}, PM₁₀, SO₂, NO₂ and PAH; that the metabolites found and identified in this study are the real cause, with PM_{2.5}, PM₁₀, SO₂, NO₂ and PAH also present; or, the most likely scenario, that a combination of the metabolites found and the consistent associations in literature are both contributing. Future studies could be done to compare the effects of PM_{2.5}, PM₁₀, SO₂, NO₂ and PAH in isolation compared to the pathways observed in this study.

Many of the chemicals identified in this study were consistent with those found in the few previous non-targeted semen studies. Sánchez-Resino et al, 2023 [21] notably identified caffeine, theophylline, several PFAS compounds, butyl-phthalates, and tributylamine. Both our study and theirs found pesticides, though different types of pesticides. This could be a result of the human geography of each of the studies, with our sample population coming from New York City and theirs coming from the LED-FERTYL program, based in Europe. Sánchez-Resino et al, 2023 had a particular focus on identifying the presence of their contaminants of note, with detection frequency as a defining characteristic in seminal plasma, whereas our study attempted to make associations between the chemicals found and specific health outcomes.

Standards were prepared (Table S1) for confirmation of 66 compounds identified at level 2 or level 3. Details of the successful level 1 identifications are included in Spreadsheet S7 in the Supporting

Information. Of the 33 that were subsequently identified as level 1, none were associated with semen health outcomes at the BH cutoff point. Four compounds did, however, have an association of $p < 0.05$ between case and control samples, with one overlap between health parameters (Table 2). No level 1 chemicals were associated with low concentration. The two associated with motility were dibutyl phthalate and 2-aminophenol. Dibutyl phthalate is a known endocrine disruptor [66], while 2-aminophenol is a cosmetic compound that has been linked to the metabolism of bisphenol F [67]. The two level 1-confidence chemicals associated with morphology were 4-nitrophenol and palmitic acid. Palmitic acid was also the lone level 1 chemical associated with low semen volume. 4-nitrophenol has been previously identified as an irritant, with prolonged exposure causing issues with blood-oxygen transport [68,69]. Palmitic acid is a common saturated fatty acid, the imbalance of which in the body can cause atherosclerosis, neurodegenerative diseases and cancer [70].

No compounds (identified or otherwise) produced a definitive association with any semen health outcome to the stringent level of BH cutoff. Many chemicals, however, were identified as having $p \leq 0.005$ when t-tested for significance with individual outcomes. Chung et al, 2019, a targeted GC-HRMS study looking for relationships between halogenated chemicals and health outcomes, were able to find similar limited associations that did not meet their correction cutoff (Bonferroni [71] rather than BH) for four types of PCB, two PFOSAs, and one PBDE [22].

Another major aim of this study was to identify health-related associations of chemicals novel to the human metabolome. The four previously unreported chemicals that have associations with semen health outcomes are compounds identified based on their InChiKey and IUPAC name. The results described in Table 3 and Figure 3 indicate that the cosmetic additive 3-hydroxyoctanedioic acid was a plausibly identified novel contributor to variance between cases and controls.

438 This study's strengths are in its approach to building combined associations between chemicals
439 and semen health parameters and its breakdown of individual chemical contributions to trends.
440 This study also does not rely on donors from fertility clinics and databases, but instead volunteers
441 from the general population, providing a more representative set of samples for New York City. The
442 unsupervised non-targeted analysis allows for the highlighting of previously under-reported
443 compounds and their unbiased associations with semen health parameters. Many other studies on
444 the impact of emerging chemicals have focused on groups of suspect compounds and their
445 potential impact on human biological systems [22,36]. In the case of this study, it allowed for the
446 uncovering of eighteen previously unreported chemicals. The use of multiple metrics of
447 association (i.e. PCA, exposure enrichment analysis) also allows for a greater number of avenues
448 and conclusions to be made when reviewing study data.

449 The first major limitation of the study is the small sample size ($n = 45$), with a relatively small
450 number of "unhealthy" participants. Sperm concentration in particular had $n = 4$ case
451 measurements that likely made a notable impact on the results for variance described in Figure 4A.
452 Other semen studies typically use sample sets in the hundreds from fertility clinic donors and
453 programs such as LED-FERTYL [72,20]. A larger sample size that still utilized the general population
454 donors of this study would have built more confidence in results and may have provided more
455 definitive associations between chemicals of interest and health parameters. A second limitation
456 is the lack of quantitative information. Though HRMS is a valuable resource for identification and
457 measurement of intensity of chemicals, the non-targeted nature of the analysis made acquisition
458 of standards for calibration and quantification of all chemicals of interest challenging. All results
459 are therefore relative, leaving out information about seminal and environmental concentrations.
460 Knowledge of concentrations could provide insight to further classify chemicals and at which
461 levels they begin to impact semen health.

The main goal of future work would be to utilize a much larger sample population (1000+ individuals) to provide definitive associations between chemicals and semen health outcomes, as per the recommendations by Chung et al, 2019 [22]. Future health outcomes would also aim to use other sample types, such as hair and urine samples as proxies to better understand the differences between the blood-prostate barrier and other blood barriers in the body, such as the blood-kidney barrier (urine) [73,74,75]. This information would also help to contextualize contaminants by showing their prevalence throughout the body's different media. Future studies would also aim to quantify chemical concentrations associated with health outcomes to help determine the levels in the body and environment at which infertility starts to become impacted.

5 Conclusions

Non-targeted analysis of semen and urine samples among men in the New York City area revealed over 7000 uniquely identified features. Eighteen compounds appear new to the human exposome when compared with the Blood Exposome Database. One of these eighteen chemicals, 3-hydroxyoctanedioic acid, was positively identified through literature spectra and had notable associations with low motility and morphology. Four level 1 identified compounds also had associations with semen health parameters: dibutyl phthalate and 2-aminophenol negatively impacted motility, 4-nitrophenol was associated with low morphology, while palmitic acid was found to be associated with both low morphology and low volume.

The collective relationship between the chemicals observed in the study and semen health parameters provide insights into the overall association between male fertility and the exposome. Though there were no single chemicals that met the minimum BH threshold for statistical significance, collective PCA analysis of all chemicals with a $p < 0.05$ relationship with each semen health parameter yielded moderate trends separating unhealthy participants from control

485 participants. Exposure enrichment analysis was used to understand the environmental sources
486 that may have influenced sperm quality. The compounds most closely related to semen health
487 outcomes yielded a notable relationship between unhealthy semen and exposure pathways
488 related to vehicle exhaust air pollution, with p-values of 3.03×10^{-4} , 2.34×10^{-5} and 5.73×10^{-4} for
489 concentration, motility and volume, respectively.

References

1. Vander Borgh, M., Wyns, C., 2018. Fertility and Infertility: Definition and Epidemiology. *Clin Biochem* 62, 2–10. doi.org/10.1016/j.clinbiochem.2018.03.012
2. Ku JY, Park NC, Jeon TG, Park HJ. Semen Analysis in Cancer Patients Referred for Sperm Cryopreservation before Chemotherapy over a 15-Year Period in Korea. *The world journal of men's health*. 2015;33(1):8-13.
3. Amirjannati N, Sadeghi M, Hosseini Jadda SH, Ranjbar F, Kamali K, Akhondi MA. Evaluation of semen quality in patients with malignancies referred for sperm banking before cancer treatment. *Andrologia*. 2011;43(5):317-320.
4. Eisenberg ML, Li S, Behr B, Pera RR, Cullen MR. Relationship between semen production and medical comorbidity. Fertility and sterility. 2015;103(1):66-71.
5. Jensen TK, Jacobsen R, Christensen K, Nielsen NC, Bostofte E. Good semen quality and life expectancy: a cohort study of 43,277 men. *American journal of epidemiology*. 2009;170(5):559-565.
6. La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. Diabetes mellitus and sperm parameters. *Journal of andrology*. 2012;33(2):145-153.
7. Salonia A, Matloob R, Gallina A, et al. Are infertile men less healthy than fertile men? Results of a prospective case-control survey. *European urology*. 2009;56(6):1025-1031.
8. Eisenberg ML, Li S, Behr B, et al. Semen quality, infertility and mortality in the USA. *Human reproduction (Oxford, England)*. 2014;29(7):1567-1574.
9. Ma, P., Zhang, Z., Zhou, X., Luo, J., Lu, H., Wang, Y., 2019. Characterizing semen abnormality male infertility using non-targeted blood plasma metabolomics. *PLoS ONE* 14, e0219179. doi.org/10.1371/journal.pone.0219179
10. Cao, T., Wang, S., Pan, Y., Guo, F., Wu, B., Zhang, Y., Wang, Y., Tian, J., Xing, Q., Liu, X., 2023. Characterization of the semen, gut, and urine microbiota in patients with different semen abnormalities. *Front Microbiol* 14, 1182320. doi.org/10.3389/fmicb.2023.1182320
11. Tong, N., Witherspoon, L., Dunne, C., Flannigan, R., 2022. Global Decline of Male Fertility: Fact or Fiction? *BC Medical Journal* 64, 1–5.
12. Jørgensen, N., Lamb, D.J., Levine, H., Pastuszak, A.W., Sigalos, J.T., Swan, S.H., Eisenberg, M.L., 2021. Are worldwide sperm counts declining? *Fertil & Ster* 116, 1457–1463. doi.org/10.1016/j.fertnstert.2021.10.020
13. Huang, B., Wang, Z., Kong, Y., Jin, M., Ma, L., 2023. Global, regional and national burden of male infertility in 204 countries and territories between 1990 and 2019: an analysis of global burden of disease study. *BMC Pub Health* 23, 2195. doi.org/10.1186/s12889-023-16793-3
14. Harris, I.D., Fronczak, C., Roth, L., Meacham, R.B., 2011. Fertility and the Aging Male. *Urol Rev* 13, e185–e190. https://doi.org/10.3909/riu0538
15. Verweij, R.M., Keizer, R., 2023. Remaining childless: examining the different patterns of expectations that lead to a childless life for men. *SN Soc Sci* 3, 60. doi.org/10.1007/s43545-023-00642-6
16. Dupree, J.M., 2016. Insurance coverage for male infertility care in the United States. *Asian J Androl* 18, 339–341. doi.org/10.4103/1008-682X.177838
17. Giwercman, A., Bonde, J.P., 1998. Declining Male Fertility and Environmental Factors. *Endocrin Metab Clin* 27, 807–830. doi.org/10.1016/S0889-8529(05)70042-6
18. Mann, U., Shiff, B., Patel, P., 2020. Reasons for worldwide decline in male fertility. *Curr Opin Urol, Infertility and Erectile Function* 30, 296–301. doi.org/10.1097/MOU.0000000000000745
19. Gautam, R., Priyadarshini, E., Patel, A.K., Arora, T., 2024. Assessing the impact and mechanisms of environmental pollutants (heavy metals and pesticides) on the male reproductive system: a comprehensive review. *J Env Sci & Health* 42, 126–153. doi.org/10.1080/26896583.2024.2302738
20. Sánchez-Resino, E., Marquès, M., Gutiérrez-Martín, D., Restrepo-Montes, E., Martínez, M.Á., Salas-Huetos, A., Babio, N., Salas-Salvadó, J., Gil-Solsona, R., Gago-Ferrero, P., 2023. Exploring the Occurrence of Organic Contaminants in Human Semen through an Innovative LC-HRMS-Based Methodology Suitable for Target and Nontarget Analysis. *Environ Sci Technol* 57, 19236–19252. doi.org/10.1021/acs.est.3c04347
21. Houle, E., Li, Y., Schroder, M., McRitchie, S.L., Tayyab, R., Sites, C.K., Sumner, S.J., Pilsner, J.R., 2023. Exploring the internal exposome of seminal plasma with semen quality and live birth: A Pilot Study. *Syst Biol Reprod Med* 69, 296–309. doi.org/10.1080/19396368.2023.2195964
22. Vitku, J., Heracek, J., Sosvorova, L., Hampl, R., Chlupacova, T., Hill, M., Sobotka, V., Bicikova, M., Starka, L., 2016. Associations of bisphenol A and polychlorinated biphenyls with spermatogenesis and steroidogenesis in two biological fluids from men attending an infertility clinic. *Environ Int* 89–90, 166–173. doi.org/10.1016/j.envint.2016.01.021
23. Chung, M.K., Louis, G.M.B., Kannan, K., Patel, C.J., 2019. Exposome-wide association study of semen quality: Systematic discovery of endocrine disrupting chemical biomarkers in fertility require large sample sizes. *Environ Intern* 125, 505–514. doi.org/10.1016/j.envint.2018.11.037

24. Marcu, D., Keyser, S., Petrik, L., Fuhrmann, S., Maree, L., 2023. Contaminants of Emerging Concern (CECs) and Male Reproductive Health: Challenging the Future with a Double-Edged Sword. *Toxics* 11, 330. doi.org/10.3390/toxics11040330
25. Feng, W., Deng, Y., Yang, F., Miao, Q., Ngien, S.K., 2023. Systematic Review of Contaminants of Emerging Concern (CECs): Distribution, Risks, and Implications for Water Quality and Health. *Water* 15, 3922. doi.org/10.3390/w15223922
26. Naidu, R., Espana, V.A.A., Liu, Y., Jit, J., 2016. Emerging contaminants in the environment: Risk-based analysis for better management. *Chemosphere* 154, 350–357. doi.org/10.1016/j.chemosphere.2016.03.068
27. Weinbuerg, N., Nelson, D., Sellers, K., Byrd, J., 2019. Insights from TSCA Reform: a Case for Identifying New Emerging Contaminants. *Curr Pollution Rep* 5, 215–227. doi.org/10.1007/s40726-019-00117-4
28. De Celis, R., Feria-Velasco, A., González-Unzaga, M., Torres-Calleja, J., Pedrón-Nuevo, N., 2000. Semen quality of workers occupationally exposed to hydrocarbons. *Fertil & Ster* 73, 221–228. doi.org/10.1016/S0015-0282(99)00515-4
29. Akhigbe, R.E., Oyedokun, P.A., Akhigbe, T.M., Adenike, S., Oladipo, A.A., Hughes, J.R., 2024. Does pyrethroid exposure lower human semen quality? a systematic review and meta-analysis. *Front Toxicol* 6. doi.org/10.3389/ftox.2024.1395010
30. Wise, C.F., Breen, M., Stapleton, H.M., 2024. Canine on the Couch: The New Canary in the Coal Mine for Environmental Health Research. *Environ Health* 2, 514–595. doi.org/10.1021/envhealth.4c00029
31. Martenies, S.E., Perry, M.J., 2013. Environmental and occupational pesticide exposure and human sperm parameters: A systematic review. *Toxicol* 307, 66–73. doi.org/10.1016/j.tox.2013.02.005
32. Osadchuk, L., Kleshchev, M., Osadchuk, A., 2023. Effects of cigarette smoking on semen quality, reproductive hormone levels, metabolic profile, zinc and sperm DNA fragmentation in men: results from a population-based study. *Front Endocrinol* 14, 1255304. doi.org/10.3389/fendo.2023.1255304
33. Whittaker, J., 2023. Dietary trends and the decline in male reproductive health. *Hormones* 22, 165–197. doi.org/10.1007/s42000-023-00431-z
34. Manz, K.E., Feerick, A., Braun, J.M., Feng, Y.-L., Hall, A., Koelmel, J., Manzano, C., Newton, S.R., Pennell, K.D., Place, B.J., Godri Pollitt, K.J., Prasse, C., Young, J.A., 2023. Non-Targeted Analysis (NTA) and Suspect Screening Analysis (SSA): A Review of Examining the Chemical Exposome. *J Exp Sci Environ Epidem* 33, 524–536. doi.org/10.1038/s41370-023-00574-6
35. Buckley, J.P., Kuiper, J.R., Bennet, D.H., Barrett, E.S., Bastain, T., Breton, C.V., Chinthakindi, S., Dunlop, A.L., Farzan, S.F., Herbstman, J.B., Karagas, M.R., Marsit, C.J., Meeker, J.D., Morello-Frosch, R., O'Connor, T.G., Romano, M.E., Schantz, S., Schmidt, R.J., Watkins, D.J., Zhu, H., Pellizzari, E.D., Kannan, K., Woodruff, T.J., 2022. Exposure to Contemporary and Emerging Chemicals in Commerce Among Pregnant Women in the United States: The Environmental Influences on Child Health Outcome (ECHO) Program. *Environ Sci Technol* 56, 6560–6573. doi.org/10.1021/acs.est.1c08942
36. Bland, G.D., Abrahamsson, D.P., Wang, M., Zlatnik, M.G., Morello-Frosch, R., Park, J.-S., Sirota, M., Woodruff, T.J., 2024. Exploring applications of non-targeted analysis in the characterization of the prenatal exposome. *Sci Tot Environ* 912, 169458. doi.org/10.1016/j.scitotenv.2023.169458
37. Hulleman, T., Turkina, V., O'Brien, J.W., Chojnacka, A., Thomas, K.V., Samanipour, S., 2023. Critical Assessment of the Chemical Space Covered by LC–HRMS Non-Targeted Analysis. *Environ Sci Tech* 57, 14101–14112. doi.org/10.1021/acs.est.3c03606
38. Cofone, L., Pindinello, I., D'Ancona, G., Grassi, F., Antonucci, A., Vitali, M., Protano, C., 2023. Human semen quality and environmental and occupational exposure to pollutants: A systematic review. *Ann Ig, Medicina Preventiva e di Comunità* 35, 660–669. doi.org/10.7416/ai.2023.2581
39. Kumar, N., Singh, A.K., 2022. Impact of environmental factors on human semen quality and male fertility: a narrative review. *Environ Sci Eur* 34. doi.org/10.1186/s12302-021-00585-w
40. Buszewska-Forajta, M., Raczak-Gutknecht, J., Struck-Lewicka, W., Nizioł, M., Artymowicz, M., Markuszewski, M., Kordalewska, M., Matuszewski, M., 2022. Untargeted Metabolomics Study of Three Matrices: Seminal Fluid, Urine, and Serum to Search the Potential Indicators of Prostate Cancer. *Front in Mol Biosci, Sec Metabolomics* 9. doi.org/10.3389/fmolb.2022.849966
41. Ulaganathan, G., Jiang, H., Canio, N., Oke, A., Armstrong, S.S., Abrahamsson, D.P., Varshavsky, J.R., Lam, J., Cooper, C., Robinson, J.F., Fung, J.C., Woodruff, T.J., Allard, P., 2024. Screening and characterization of 133 physiologically-relevant environmental chemicals for reproductive toxicity. *Reprod Tox* 126, 108602. doi.org/10.1016/j.reprotox.2024.108602
42. Samuel, P.O., Edo, G.I., Oloni, G.O., Ugbune, U., Ezekiel, G.O., Essaghah, A.E.A., Agbo, J.J., 2023. Effects of chemical contaminants on the ecology and evolution of organisms a review. *Chem & Ecol* 39, 1071–1107. doi.org/10.1080/02757540.2023.2284158

43. Johnson, T.A., Abrahamsson, D.P., 2024. Quantification of chemicals in non-targeted analysis without analytical standards – Understanding the mechanism of electrospray ionization and making predictions. *Curr Opin Environ Sci Health* 37, 100529. doi.org/10.1016/j.coesh.2023.100529
44. Guo, Z., Huang, S., Wang, J., Feng, Y.-L., 2020. Recent advances in non-targeted screening analysis using liquid chromatography - high resolution mass spectrometry to explore new biomarkers for human exposure. *Talanta* 219, 121339. doi.org/10.1016/j.talanta.2020.121339
45. Tkalec, Ž., Codling, G., Klánová, J., Horvat, M., Kosjek, T., 2022. LC-HRMS based method for suspect/non-targeted screening for biomarkers of chemical exposure in human urine. *Chemosphere* 300, 134550. doi.org/10.1016/j.chemosphere.2022.134550
46. CompTox Chemicals Dashboard: Release Notes, Apr 2024. United States Environmental Protection Agency.
47. Barupal DK, Fiehn O. Generating the blood exposome database using a comprehensive text mining and database fusion approach. *Environ health perspect.* 2019 Sep 26;127(9):097008. doi.org/10.1289/EHP4713
48. PubChem. National Center for Biotechnology Information. Available at: <https://pubchem.ncbi.nlm.nih.gov/> (Last accessed: 21, Aug, 2024).
49. Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Viau, C., Spigelman, A.F., MacDonald, P.E., Wishart, D.S., Li, S., Xia, J., 2024. MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. *Nucleic Acids Research* gkae253. doi.org/10.1093/nar/gkae253
50. Automated Self-Administered 24-Hour (ASA24®) Dietary Assessment Tool, 2024. <https://epi.grants.cancer.gov/asa24/>
51. WHO Laboratory Manual for the Examination and Processing of Human Semen, 6th ed, 2021. <https://iris.who.int/bitstream/handle/10665/343208/9789240030787-eng.pdf?sequence=1>
52. Alshahrani, S., Aldossari, K., Al-Zahrani, J., Gabr, A.H., Henkel, R., Ahmad, G., 2017. Interpretation of Semen Analysis using WHO 1999 and WHO 2010 Reference Values: Abnormal Becoming Normal. *Andrologia* 50, e12838. doi.org/10.1111/and.12838
53. Tsugawa, H., Ikeda, K., Takahashi, M., Satoh, A., Mori, Y., Uchino, H., Okahashi, N., Yamada, Y., Tada, I., Bonini, P., Higashi, Y., Okazaki, Y., Zhou, Z., Zhu, Z.-J., Koelmel, J., Cajka, T., Fiehn, O., Saito, K., Arita, Masanori, Arita, Makoto, 2020. A lipidome atlas in MS-DIAL 4. *Nat Biotechnol* 38, 1159–1163. doi.org/10.1038/s41587-020-0531-2.
54. Sumner, L.W., Amberg, A., Barrett, D., Beale, M.H., Beger, R., Daykin, C.A., Fan, T.W.-M., Fiehn, O., Goodacre, R., Griffin, J.L., Hankemeier, T., Hardy, N., Harnly, J., Higashi, R., Kopka, J., Lane, A.N., Lindon, J.C., Marriott, P., Nicholls, A.W., Reilly, M.D., Thaden, J.J., Viant, M.R., 2007. Proposed minimum reporting standards for chemical analysis. *Metabolomics* 3, 211–221. doi.org/10.1007/s11306-007-0082-
55. Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J Roy Stat Soc, Methodological* 57, 289–300. doi.org/10.1111/j.2517-6161.1995.tb02031.x
56. Djoumbou Feunang, Y., Eisner, R., Knox, C., Chepelev, L., Hastings, J., Owen, G., Fahy, E., Steinbeck, C., Subramanian, S., Bolton, E., Greiner, R., Wishart, D.S., 2016. ClassyFire: Automated Chemical Classification with a Comprehensive, Computable Taxonomy. *J Cheminform* 8, 61. doi.org/10.1186/s13321-016-0174-y
57. Vitku, J., Heracek, J., Sosvorova, L., Hampl, R., Chlupacova, T., Hill, M., Sobotka, V., Bicikova, M., Starka, L., 2016. Associations of bisphenol A and polychlorinated biphenyls with spermatogenesis and steroidogenesis in two biological fluids from men attending an infertility clinic. *Environ Int* 89–90, 166–173. doi.org/10.1016/j.envint.2016.01.021
58. Galimova, E.F., Amirova, Z.K., Galimov, S.N., 2015. Dioxins in the semen of men with infertility. *Environ Sci Pollut Res* 22, 14566–14569. doi.org/10.1007/s11356-014-3109-z
59. Song, X., Zhuo, Q., Tang, S., Xie, T., Chen, Z., Zang, Z., Zhang, Y., Niu, X., Yin, H., Zeng, F., He, C., 2020. Concentrations of phthalates metabolites in blood and semen and the potential effects on semen concentration and motility among residents of the Pearl River Delta region in China. *Emerg Contam* 6, 39–43. <https://doi.org/10.1016/j.emcon.2019.12.002>
60. Di Nisio, A., Sabovic, I., Valente, U., Tescari, S., Santa Rocca, M., Guidolin, D., Dall’Acqua, S., Acquasaliente, L., Pozzi, N., Plebani, M., Garolla, A., Foresta, C., 2018. Endocrine Disruption of Androgenic Activity by Perfluoroalkyl Substances: Clinical and Experimental Evidence. *J Clin Endocrin Metab* 104, 1259–1271. doi.org/10.1210/jc.2018-01855
 - a. Corrigendum to: “Endocrine Disruption of Androgenic Activity by Perfluoroalkyl Substances: Clinical and Experimental Evidence”, The Journal of Clinical Endocrinology & Metabolism, Volume 106, Issue 6, June 2021, Page e2463, <https://doi.org/10.1210/clinem/dgab253>
61. Wang, A., Abrahamsson, D.P., Jiang, T., Wang, M., Morello-Frosch, R., Park, J.-S., Sirota, M., Woodruff, T.J., 2021. Suspect Screening, Prioritization, and Confirmation of Environmental Chemicals in Maternal-Newborn Pairs from San Francisco. *Ecotoxicol Environ Saf* 55, 4183–5602. doi.org/10.1021/acs.est.0c05984

62. Richter, L., Corder, A., Brown, P., 2020. Producing Ignorance Through Regulatory Structure: The Case of Per- and Polyfluoroalkyl Substances (PFAS). *Sociol Perspect* 64, 631. <https://doi.org/10.1177/0731121420964827>
63. Xu, R., Zhong, Y., Li, R., Li, Y., Zhong, Z., Liu, T., Wang, Q., Lv, Z., Huang, S., Duan, Y.-G., Zhang, X., Liu, Y., 2023. Association between exposure to ambient air pollution and semen quality: A systematic review and meta-analysis. *Sci Tot Environ* 870, 161892. doi.org/10.1016/j.scitotenv.2023.161892
64. Liu, J., Dai, Y., Li, J., Wang, Q., Wang, L., 2023. Does air pollution exposure affect semen quality? Evidence from a systematic review and meta-analysis of 93,996 Chinese men. *Front Pub Health* 11, 1219340. doi.org/10.3389/fpubh.2023.1219340
65. Rubes, J., Selevan, S.G., Sram, R.J., Evenson, D.P., Perreault, S.D., 2007. GSTM1 genotype influences the susceptibility of men to sperm DNA damage associated with exposure to air pollution. *Mutat Res Fundam Mol Mech Mutagen* 625, 20–28. doi.org/10.1016/j.mrfmmm.2007.05.012
66. Czubacka, E., Czerczak, S., Kupczewska-Dobacka, M.M., 2021. The overview of current evidence on the reproductive toxicity of dibutyl phthalate. *Int J Occup Med Environ Health* 34, 15–37. doi.org/10.13075/ijom.1896.01658
67. Tchen, R., Tan, Y., Barr, D.B., Ryan, P.B., Tran, V., Li, Z., Hu, Y.-J., Smith, A.K., Jones, D.P., Dunlop, A.L., Liang, D., 2022. Use of high-resolution metabolomics to assess the biological perturbations associated with maternal exposure to Bisphenol A and Bisphenol F among pregnant African American women. *Environ Intern* 169, 107530. doi.org/10.1016/j.envint.2022.107530
68. Serrà, A., Artal, R., Garcia-Amorós, J., Gómez, E., 2020. Simple Environmentally-Friendly Reduction of 4-Nitrophenol. *Catalysts* 10, 458. doi.org/10.3390/catal10040458
69. Yeh, C.-C., Chen, D.-H., 2014. Ni/reduced graphene oxide nanocomposite as a magnetically recoverable catalyst with near infrared photothermally enhanced activity. *Appl Catal B Environ* 150–151, 298–304. doi.org/10.1016/j.apcatb.2013.12.040
70. Carta, G., Murru, E., Banni, S., Manca, C., 2017. Palmitic Acid: Physiological Role, Metabolism and Nutritional Implications. *Front Physiol* 8. doi.org/10.3389/fphys.2017.00902
71. Dunn OJ. Multiple comparison among means. *J Am Stat Assoc* 1961; 56: 52–64. doi.org/10.1080/01621459.1961.10482090
72. Ren, M., Fang, M., Liu, J., Lu, Q., Bao, H., Zhuang, L., Meng, F., Pan, B., Yan, L., Li, Z., Xu, J., Han, B., Bai, Z., Tian, C., Wang, Y., Wang, B., 2024. Applying hair exposome for linking environmental exposure to reproductive health: A comprehensive review and research perspective. *Hyg Environ Health Adv* 9, 100086. doi.org/10.1016/j.heha.2023.100086
73. Blaurock, J., Baumann, S., Grunewald, S., Schiller, J., Engel, K.M., 2022. Metabolomics of Human Semen: A Review of Different Analytical Methods to Unravel Biomarkers for Male Fertility Disorders. *Int J Mol Sci* 23, 9031. doi.org/10.3390/ijms23169031
74. Longo, V., Forleo, A., Ferramosca, A., Notari, T., Pappalardo, S., Siciliano, P., Capone, S., Montano, L., 2021. Blood, urine and semen Volatile Organic Compound (VOC) pattern analysis for assessing health environmental impact in highly polluted areas in Italy. *Environ Poll* 286, 117410. doi.org/10.1016/j.envpol.2021.117410
75. Menon, M.C., Chuang, P.Y., He, C.J., 2012. The Glomerular Filtration Barrier: Components and Crosstalk. *Int J Nephrol* 2012, 749010. doi.org/10.1155/2012/749010