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Research Article

Multi-matrix chemical exposome characterization and its association with semen quality

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Abstract

There is strong evidence to suggest that the chemical exposome may have an impact on human spermatogenesis, contributing to the male fertility decline observed during the last decades. The present study was aimed at identifying the presence of organic chemicals in human seminal plasma, blood plasma and urine, and explore their associations with seminal quality parameters. We applied a novel LC-HRMS based chemical profiling approach for screening more than 2000 organic chemicals in these biofluids from participants (n = 48) to the Spanish Led-Fertyl cohort. The results showed that 42, 42 and 48 chemicals were respectively detected in at least one sample of seminal plasma, urine, and blood plasma, respectively. These co-exposure mixtures included artificial sweeteners, biocides, flame retardants, food-related compounds, PFAS, pharmaceuticals and biomarkers of tobacco exposure. Up to 26 chemicals were detected in all three matrices with positive correlations between them. In turn, our results highlight seminal plasma as a valuable matrix for exposure assessment, as certain chemicals were detected exclusively in this biospecimen, while others—although also present in blood plasma and urine—reached their highest concentrations in seminal plasma. Statistical analyses revealed negative associations between several lesser-studied chemicals—such as 1,3-Dicyclohexylurea, propylparaben, bisphenol S or the biocide nitenpyram—and multiple semen quality parameters. In addition, our approach also confirmed the known negative associations with PFAS and smoking-related compounds, including nicotine and cotinine. This study underscores the potential of wide-scope targeted approaches in epidemiological research, providing valuable insights into the complex interplay between chemical exposures and reproductive health.

Key words: chemical exposome; LC-HRMS; semen quality; male fertility; environmental exposures; exposure-response associations.

Introduction

The global trends of human population growth have undergone an alarming switch over the last five decades, characterized by a persistent decline in fertility rates worldwide, but particularly in the most developed and industrialized countries.[1] Deterioration in sperm quality is one of the best-documented biological factors paralleling these downward trends in fertility. For instance, a meta-analysis of 185 studies involving 42 935 men showed a significant overall decline in sperm concentrations of 52.4% between 1793 and 2011 in Western men.[2] A list of lifestyle and environmental factors have been suspected of playing a role in sperm quality alterations including smoking, poor diet, low physical activity and exposure to chemical toxicants.[3]

Modern lifestyles have raised the number of synthetic chemicals released to the environment and, consequently, to which humans are exposed throughout their life.[4] Some of these may mimic or alter the normal hormonal function, so-called endocrine-disrupting chemicals (EDCs), which are increasingly linked to human diseases.[5] Prenatal exposure to EDCs has been associated to male reproductive disorders across the lifespan, supporting the testicular dysgenesis syndrome hypothesis.[6] Some studies have also linked adult exposure to individual EDCs such as phthalates, bisphenol A and certain persistent organic pollutants to poor semen quality, but the overall evidence remains limited and often inconsistent.[7,8] Recent studies have emphasized the need of characterizing chemicals as mixtures beyond

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the reductionist single-pollutant approach to better capture real-world exposures and their cumulative impact on male fertility. In fact, multiple molecular initiating events and key events may converge downstream to impair sperm quality, indicating that diverse environmental chemicals may interact as components of toxicologically active mixtures.[9,10] This convergence underscores the need for chemical risk assessment frameworks to take into account the combined effects of several agents rather than evaluating substances in isolation.

The incremental number of chemicals released in the environment annually combined with the novel developments in high-resolution mass-spectrometry (HRMS), has boosted the development of both multi-targeted, suspect screening and non-targeted screening strategies in human biomonitoring to identify in particular those chemicals of emerging concern.[4,11,12] Despite considerable efforts in the field of exposomics, the chemical exposome–understood as the totality of chemicals an individual encounters throughout their lifetime and their interactions within biological systems–remains largely unexplored in biological matrices, requiring tailored analytical strategies combining targeted and non-targeted methods to achieve a suitable balance between sensitivity and chemical coverage.[13,14] Comprehensive multi-targeted methods have been proposed as good alternatives for exposome-scale studies, offering a good analytical compromise, overcoming problems related to structural identification, providing quantitative measurements and being scalable to large-scale epidemiological settings. The large potential of multi-targeted approaches based on liquid chromatography coupled to HRMS has been illustrated in different biological matrices including urine,[15] blood[16] and seminal plasma,[17] allowing for the comprehensive quantitative analysis of over 2000 chemicals.

The aim of the present study was a) to explore the chemical exposome in paired samples of seminal plasma, blood plasma and urine from healthy men and b) to characterize their associations with semen quality parameters.

Methods

Study population

The study population was selected from the Led-Fertyl cross-sectional study *"Lifestyle and Environmental Determinants of Seminogram and other Male Fertility-related Parameters,"* a cohort of healthy men aged 18 - 40 years established between February 2021 and April 2023. Recruitment details have been described in detail elsewhere.[18,19] Briefly, the exclusion criteria accounted for severe chronic illnesses, reproductive disorders, major organ transplants, cardiovascular disease, HIV or hepatitis B/C, active or recent cancer, severe psychiatric or endocrine disorders, liver failure, use of certain medications (like antidepressants, corticosteroids, or immunosuppressants), significant recent weight loss, and any condition that might hinder adherence to the study protocol. The present study included 48 participants randomly selected from the pool of 200 participants with available biological samples and measured outcomes. All procedures followed were in accordance with the ethical standards of the Helsinki Declaration. The project protocol received approval from the Ethics Committee of the Pere Virgili Health Research Institute (Ref. CEIM: 181/2019). An online and written informed consent was provided from all participants.

Biological sample collection

Blood, urine and semen samples were collected during the same morning visit at the Hospital Universitari Sant Joan de Reus (Reus, Tarragona, Spain). The details for the biocollection have been described elsewhere.[17,19] Fasting spot urine samples were stored in aliquots in polypropylene tubes and kept at −80°C until the laboratory analysis. Semen samples were collected following a minimum of 3 days of sexual abstinence, with the P25-P75 range being 3–5 days. Samples were obtained through masturbation and deposited into sterile, standard polypropylene containers. After a 20-min liquefaction period at 37°C, semen quality parameters and chemical exposome were analysed. Venous blood samples were collected after a minimum fasting period of 8 h into 10 mL Vacutainer tubes containing lithium heparin as the anticoagulant, and were also kept at −80°C until the laboratory analysis.

Semen quality parameters

Macroscopic parameters, including semen volume and pH, were assessed. Microscopic examination involved the use of a phase-contrast microscope and a computer-assisted sperm analysis (CASA) system SCA, Microptic, version 6.5.0.67 (Microptic, Barcelona, Spain). This comprehensive analysis covered conventional factors such as sperm count and concentration, sperm motility, sperm vitality, and sperm morphology. Collection and examination procedures adhered to the World Health Organization standards (WHO, 2021).[20] Briefly, sperm count, and concentration were measured with the 10× phase contrast objective and expressed as millions of spermatozoa per ejaculate or millions of sperm cells per mL, respectively. Sperm motility was evaluated in 200 spermatozoa by analysing various real-time images captured by the CASA system, with each sperm cell categorized as progressive motile, non-progressive motile, or immotile. Motility was further quantified as a percentage of the total motility observed, encompassing both progressive and non-progressive motility. Sperm vitality was assessed using the hypo-osmotic swelling test (HOS test) at 60× magnification and analysing 200 sperm cells. Additionally, sperm morphology was examined utilizing the Hemacolor (Millipore, Sigma-Aldrich, Darmstadt, Germany) staining protocol, observing 200 sperm cells under 60× brightfield optics. Morphology assessment involved quantifying the percentage of normal forms or abnormalities in the head, midpiece, terminal piece or combined abnormalities.

Chemicals, reagents, and analyte selection

Analytical-grade solvents including methanol (MeOH, HPLC-grade), acetonitrile (ACN, HPLC-grade), water (HPLC-grade), formic acid (>99% purity), ammonium acetate, and ammonium formate (≥99.0% purity) were obtained from Merck (Darmstadt, Germany). Ultrapure water was produced using a Milli-Q purification system (Aurium, PRO-VFT, Sartorius, Göttingen, Germany). Captiva 3 mL Non-Drip filter cartridges were obtained from Agilent (Madrid, Spain). Analytical standards and isotopically labeled internal standards (ISs), were purchased from LGC Standards (Teddington, UK), Merck (Darmstadt, Germany), and Sigma-Aldrich (St Louis, MO, USA).

A comprehensive chemical database was employed for wide-scope targeted screening (Table S1), previously applied in human biomonitoring studies in urine, blood plasma, and semen.[15-17]. The database was obtained from the Bruker TargetScreener method (Bruker Daltonics, Bremen, Germany) and was complemented with additional in-house compounds, including MS/MS information and retention times (RT). The final database comprises 2029 individual compounds covering the following major chemical classes(subclasses): artificial sweeteners, industrial

chemicals & transformation products (TPs) (eg, PFAS, flame retardants, plasticizers, benzothiazoles, benzotriazoles, surfactants, UV stabilizers or tyre additives), naturally occurring substances & stimulants & TPs (eg, food-derived, or tobacco-derived), personal care products & TPs (eg, UV-filters), pharmaceuticals (PhACs) & TPs, and plant protection products & TPs (eg, biocides, or preservatives). Analytes were selected based on their potential presence in the aforementioned biological matrices.

Wide-scope chemical exposome profiling in biological matrices

Previously validated methods have been used to conduct a chemical exposome profiling of more than 2000 compounds after minimal sample preparation, ensuring the preservation of a maximal number of chemicals of interest.[15,17] Briefly, simplified sample preparation was performed for the three matrices as follows: semen samples were thawed at room temperature, incubated at 37°C for 15 min to achieve liquefaction, and centrifuged (10 000 g, 10 min) to obtain seminal plasma. An aliquot of the supernatant (100 μL) was mixed with 300 μL acetonitrile for protein precipitation and centrifuged again (10 000 g, 10 min) before transfer to a chromatographic vial. Blood serum/plasma samples were thawed, 200 μL aliquots were mixed with 600 μL acetonitrile, vortexed, centrifuged (10 000 g, 10 min), and the supernatant transferred to chromatographic vials. Urine samples were centrifuged (3500 rpm, 5 min), filtered through Captiva cartridges (Agilent, Madrid, Spain), and 475 μL of filtrate mixed with 25 μL methanol in a vial. Sample extracts were injected in an ultrahigh-performance liquid chromatography (UHPLC) system with a Bruker Elute Pump HPG 1300 coupled to a QTOF Impact II (Bruker, Bremen, Germany). The chromatographic separation was performed on an Intensity Solo column (2.1 × 100 mm, 1.8 μm) from Bruker, preceded by a guard column, CORTECTS C18, 1.7 μm (2.1 × 5) mm from Waters (Milford, USA), thermostated in an air oven at 40°C. Instrument was operated in broadband collision-induced dissociation (bbCID), a data-independent acquisition (DIA) mode, at 3 scans per second. The aqueous mobile phase consisted of H2O: MeOH (99:1) with solvent modifiers consisting in 5 mM ammonium formate and 0.01% formic acid for ESI(+), and 5 mM ammonium acetate for ESI(−). The organic phase was MeOH with identical additives for each ionization mode, respectively.

Concerning quantification, matrix-matched calibration curves were used for chemicals with available standards. Matrix-matched calibration curves were prepared for each matrix under identical experimental conditions as the samples to ensure reproducibility and comparability of the results. For compounds without available matrix-matched calibration curves, semi-quantification was performed using a quantitative structure–ionization relationship (QSIR) model based on ionization efficiency (IE).[15,21] This approach uses a log-transformed ratio of calibration curve slopes relative to reference compounds [bisphenol G for ESI (−), O-desmethyl venlafaxine for ESI(+)], normalized to reflect ionization potential across compounds. Table S3 presents the chemicals used to construct the matrix-matched calibration curve employed in the semi-quantification model. To account for matrix effects, a limited number of calibrant compounds were projected onto the biological matrix. The pseudo-molecular ions ($[M-H]^-$ and $[M+H]^+$) were used as diagnostic signals to calculate peak areas and logIE values. For these semi-quantified compounds, limits of detection (LOD) were estimated based on a signal-to-noise ratio approach, defined as three times the noise level (LOD = 3×S/N), using the signal in sample.

Quality assurance and quality control (QA/QC)

Strict QA/QC protocols were implemented to ensure data integrity and minimize contamination or instrumental bias throughout sample processing and analysis. All glassware was cleaned with Milli-Q water and acetone, and baked at 450 °C for 6 h prior to use. Work surfaces were decontaminated daily using water and acetone. A total of 26 procedural blanks—accounting for ∼18% of the total sample number—were processed using HPLC-grade water instead of biological matrix, and subjected to identical extraction and analysis procedures. Blank signals plus three times the standard deviation were subtracted from sample peak areas prior to quantification to correct for background contamination. Instrumental performance was monitored by injecting methanol and a 50 ng/mL standard mixture every 20 injections. Samples were injected in a randomized sequence, including first blood plasma, then seminal plasma, and finally urine samples. Clothianidin-d_3 was added before extraction to monitor potential sample preparation losses, while an internal standard (IS) mixture was added prior to LC-HRMS analysis to correct for matrix effects and instrumental fluctuations. IS list is provided in Table S3. As the coefficient of variation (CV%) for all internal standards (IS) remained low along the batch (average <12%), the analyte areas were corrected using the mean IS area per sample, providing a robust general correction in the absence of compound-specific IS (Table S3). To ensure high mass accuracy, external calibration using sodium formate was performed before each analytical batch. Additionally, internal calibration was carried out in the first 15s of each run using sodium formate/acetate in a 2-propanol/water mixture (50:50, v/v), allowing real-time mass correction.

Statistical analysis

Descriptive analysis was conducted for demographics, individual and semen parameter data. Partition coefficients between biological matrices (seminal plasma/blood plasma and seminal plasma/urine) were calculated as the ratio of median concentrations for each detected compound. Chemicals with a detection frequency above 75% were used as continuous variables in regression analysis substituting the non-detected observations by the limit of detection (LOD)/$\sqrt{2}$.[22] Chemicals detected between 10% and 75% of samples were modeled in regression models as binary variables (detected Versus non-detected). Spearman correlation analysis was conducted to characterize the bivariate associations between chemicals in the same biological matrix and between matrices. Multiple linear regression was conducted to characterize the associations between semen quality parameters (log-transformed) and chemical concentrations (log-transformed) or presence (binary) as independent variables. The models were adjusted by confounding variables selected a priori using a minimally adjusted causal structure considering age (continuous, years), abstinence (continuous, days) and smoking (binary). A set of sensitivity analysis were conducted to assess the influence of physical activity (Metabolic Equivalent of Task (MET), continuous, MET min/week), body mass index (continuous, kg/m^2), professional activity or residence in the proximity of a petrochemical industry in the results (binary). Due to the limited sample size and the exploratory nature of the study, the results from regression analysis were not adjusted by false discovery proportion. In order to discern potential co-exposure patterns or interactions, multipollutant models were built using gradient boosting trees allowing to combine continuous and binary exposure variables. We implemented the method using the R packages “gbm” and “dismo.” The gbm.step function was

computed using the input parameters setup 0.001 for the learning rate and 4 for the tree complexity, the 0.8 bag fraction at 0.8, and a 10-fold cross-validation. We computed a two-step approach considering first a full model with all variables followed up by reduced model with a subset of variables with contributions above 2%. The relative contribution of biomarkers to the overall model fit was used as a variable importance metric and the marginal effects were inspected with the partial dependency plots. All statistical analysis were conducted with R software v. 4.2.3.[23]

Results

The 48 men selected in the present analysis had a mean (SD) age of 32 (4) years and BMI of 26 (3) kg/m^2 (Table 1). Ten of them (21%) reported to be current or past smokers. In average, they reported a consumption of 11 g alcohol/day, and the mean physical activity was 3321 (1940) MET min/week. The median abstinence was 4 days, and a low proportion of participants showed abnormal semen parameters ranging between 4% and 8% for most parameters except for sperm morphology, abnormal among 20% of participants. The characteristics of the Led-Fertyl subset evaluated in the present study were very similar to the rest of Led-Fertyl participants (Table S4). The sociodemographic variables showed globally null associations with semen quality parameters, with the exception of physical activity and living close to a petrochemical industry, slightly positively associated with form and concentration, respectively (details in Figure S1).

Chemical exposome profiling of seminal plasma, blood plasma and urine

The wide-scope target screening method enabled the identification of 61 chemicals in at least one sample across the three analyzed matrices including a variety of chemical families such as artificial sweeteners, biocides, flame retardants, food-derived compounds, PFAS, pharmaceuticals or tobacco smoke chemicals. The detection frequencies (%DF), limits of quantification (LOQ), median concentrations and whether concentrations were determined by quantification (n_{serum}= 12; $n_{seminal\ plasma}$= 15; n_{urine}= 15) or semi-quantification (n_{serum}= 30; $n_{seminal\ plasma}$= 30; n_{urine}= 34) of all detected chemical are summarized in Figure 1 and supplemental Table S5. The average blanks concentration was also expressed in Table S5. The number of chemicals frequently detected (eg, above 75% of samples) ranged between 9 in seminal plasma or urine and 15 in plasma. The majority of chemicals were detected in less than 25% of analyzed samples.

Twenty-six chemicals were found in the three matrices (Figure S2), and the number of chemicals identified in just one matrix ranged between 4 (blood plasma) and 10 (urine). Strong positive correlations between matrices were observed for most chemicals, particularly for tobacco smoke compounds (See diagonal in the correlation heatmaps of supplemental Figures S3-S5). In turn, some chemicals showed no association or even mild negative correlations, highlighting the specificity of the matrix. That is the case for instance of mono(2-ethylhexyl) phthalate (MEHP) or mono n/isobutyl phthalate (MxBP).

The average partition coefficient of chemicals between matrices showed an overall trend to concentrate in seminal plasma over urine, mostly in the range 10 - 100 fold, but in some cases, such as quinmerac and nicotinamide, that exceeded a 100-fold concentration in seminal plasma (supplemental Figure S6). In case of the ratio seminal plasma/plasma we observed a large proportion of chemicals with a ratio below the unit (ie, indicating higher concentrations in plasma) including perfluorooctanesulfonic acid (PFOS), MEHP or hydroxycotinine.

Table 1. Study population characteristics (n = 48).

Demographic characteristics	Overall (N = 48)
Age (years)	31.7 (4.3)
Body mass index (kg/m^2)	25.5 (2.8)
Current or past smokers (n [%])	10 (21%)
Physical activity (MET min/week)	3321 (1940)
Alcohol consumption (g/day)	10.9 (8.7)
Semen parameters [% of abnormal]	
Volume (mL)	3.4 (2.4-4.1) [4]
Abstinence (days)	4.0 (3.0 - 5.0)
Total sperm count (×10^6 spz)	209.8 (116.9 - 347.7) [4]
Sperm concentration (×10^6 spz/mL)	61.6 (33.0 - 95.6) [8]
Sperm vitality (%)	80.0 (73.8 - 82.6) [6]
Total sperm motility (%)	64.5 (54.0 - 72.4) [8]
Normal sperm morphology (%)	8.0 (4.4 - 16.9) [21]

Demographic variables are expressed as mean (standard deviation). Semen parameters are expressed as median (Q1-Q3).
Normal seminogram WHO standards: Sample volume ≥ 1.4 mL, pH between 7.2 and 8.0, total sperm count > 39 million, sperm concentration ≥ 16 millions/mL, sperm vitality > 54%, total motility ≥ 30%, normal form sperm ≥ 4% (WHO, 2021).

Associations between chemicals and semen quality parameters

The multivariate regression analysis adjusting for covariates (abstinence, age and smoking) showed a wide variety of chemical families systematically associated with multiple parameters of poor sperm quality. These included the artificial sweetener acesulfame, biocides (nitenpyram or 1,2-Benzisothiazol-3(2H)-one [BIT]), personal care products (propyl- and methylparabens), the plasticizer bisphenol S (BPS), the surfactant N, N-Dimethyldodecylamine (DMDDA), the tire additive 1,3-dicyclohexylurea (DCU) and a list of tobacco smoke compounds (Figure 2). While tobacco smoke compounds showed strong correlations among matrices, the associations with semen quality parameters were substantially strengthened when exposures were assessed in seminal plasma. Some chemicals showed isolated statistical associations with individual semen parameters or in specific matrices. For instance, the presence of PFOS in blood plasma was negatively associated with seminal volume or pentachlorophenol (PCP) showed a negative association with the normal morphology. We also identified some food-derived chemicals positively associated with multiple semen quality parameters across different matrices including nicotinamide (vitamin B3 derivative) or theophylline.

Multipollutant gradient-boosting tree (GBT) models showed complex associations between chemicals with multiple semen quality parameters. The analysis reveals that chemical mixtures exhibit both individual and interactive effects on sperm function, with variable importance and directionality differing across endpoints (Figure 3 and supplemental Figures S10-S12). Several interactions substantially contributed to the models for sperm concentration, total motility, count and vitality, indicating that chemical co-exposures may be more important than individual chemical effects for these endpoints. Nicotinamide appeared to be one of the most influential factors for most models, interacting with multiple chemicals. Pentachlorophenol (PCP) appeared to be negatively associated to total motility, normal form and vitality, whereas negative contributions of PFOA or PFOS were revealed for total motility, normal form, volume or vitality. DCU appeared to be the top ranked chemical negatively associated to vitality, interacting with paraxanthine and caffeine. Overall,

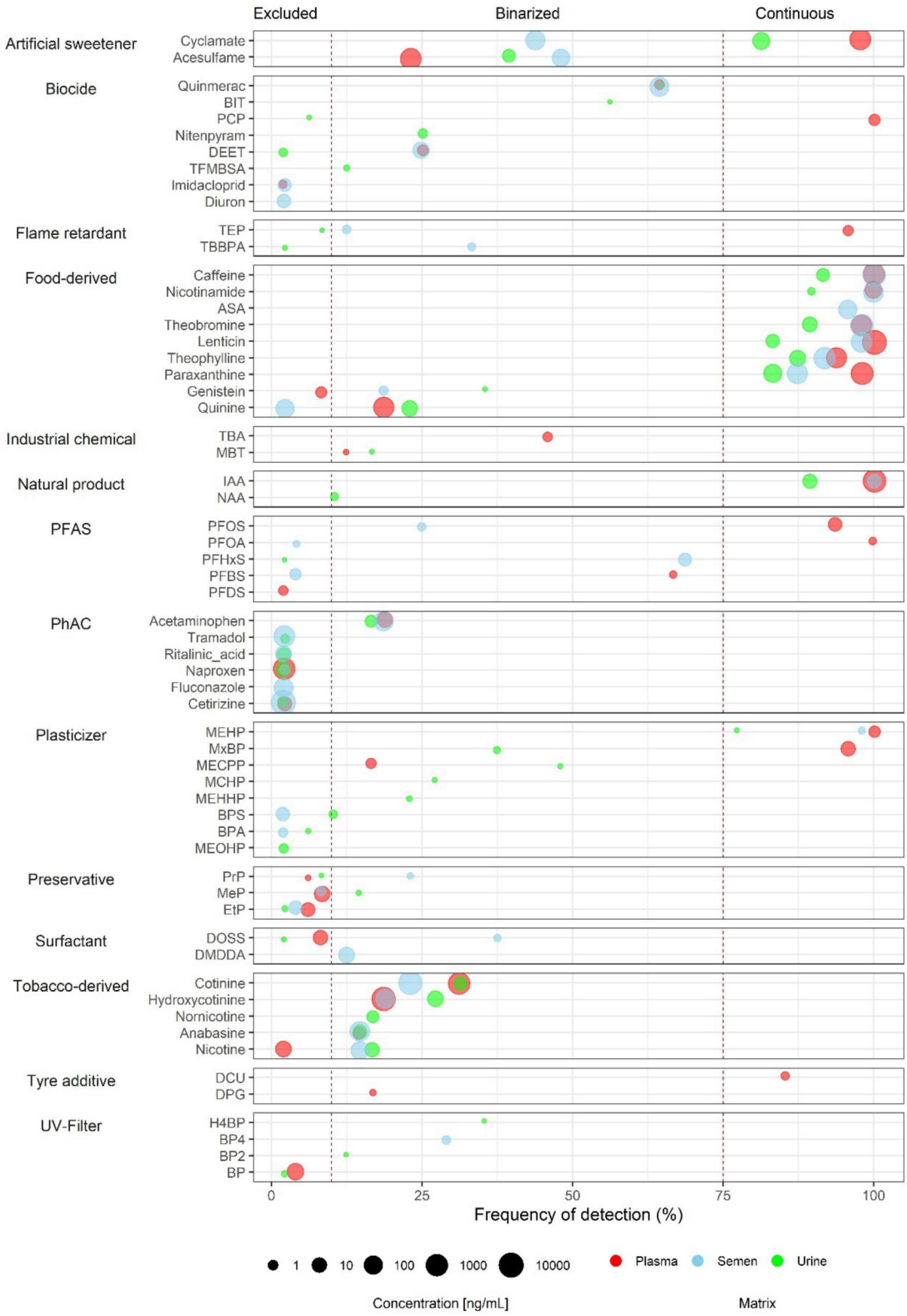

Figure 1. Bubble plot depicting the detection frequencies and concentrations of chemicals in seminal plasma (blue), urine (green) and blood plasma (red) from Led-Fertyl participants (n = 48). Bubble size corresponds to median concentration levels (ng/mL). The dotted red lines depict the lower bounds used as a threshold to select those chemicals used as continuous variables (75%) and binary variables (10%) in regression models. Abbreviations: ASA; 5-Aminosalicylic acid, BIT; 1,2-Benzisothiazol-3(2H)-one, BP; Benzophenone, BP2; 4,4′-Dihydroxybenzophenone, BP4; Benzophenone-4, BPA; Bisphenol A, BPS; Bisphenol S, DCU; 1,3-Dicyclohexylurea, DEET; Diethyltoluamide, DMDDA; N, N-Dimethyldodecylamine, DOSS; Dioctyl sulfosuccinate, DPG; N, N′-Diphenylguanidine, EtP; Ethylparaben, H4BP; 4-Hydroxybenzophenone, IAA; Indole-3-acetic acid, MBT; 2-Mercaptobenzothiazole, MCHP; Mono-cyclohexyl phthalate, MECPP; Mono(5-carboxy-2-ethylpentyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MeP; Methylparaben, MxBP; Mono-n-butyl phthalate/ Mono-isobutyl phthalate, NAA; 1-Naphthaleneacetic acid, PCP; Pentachlorophenol, PFBS; Perfluorobutanesulfonic acid, PFDS; 1H, 1H, 2H, 2H-Perfluorodecanesulphonic acid, PFHxS; Perfluorohexanesulfonic acid, PFOA; Perfluorooctanoic acid, PFOS; Perfluorooctanesulfonic acid, PrP; Propylparaben, TBA; Tributylamine, TBBPA; Tetrabromobisphenol A, TEP; Triethyl phosphate, TFMBSA; 2-(Trifluoromethyl)benzenesulfonamide. Details on abbreviations are also available in supplemental Table S6.

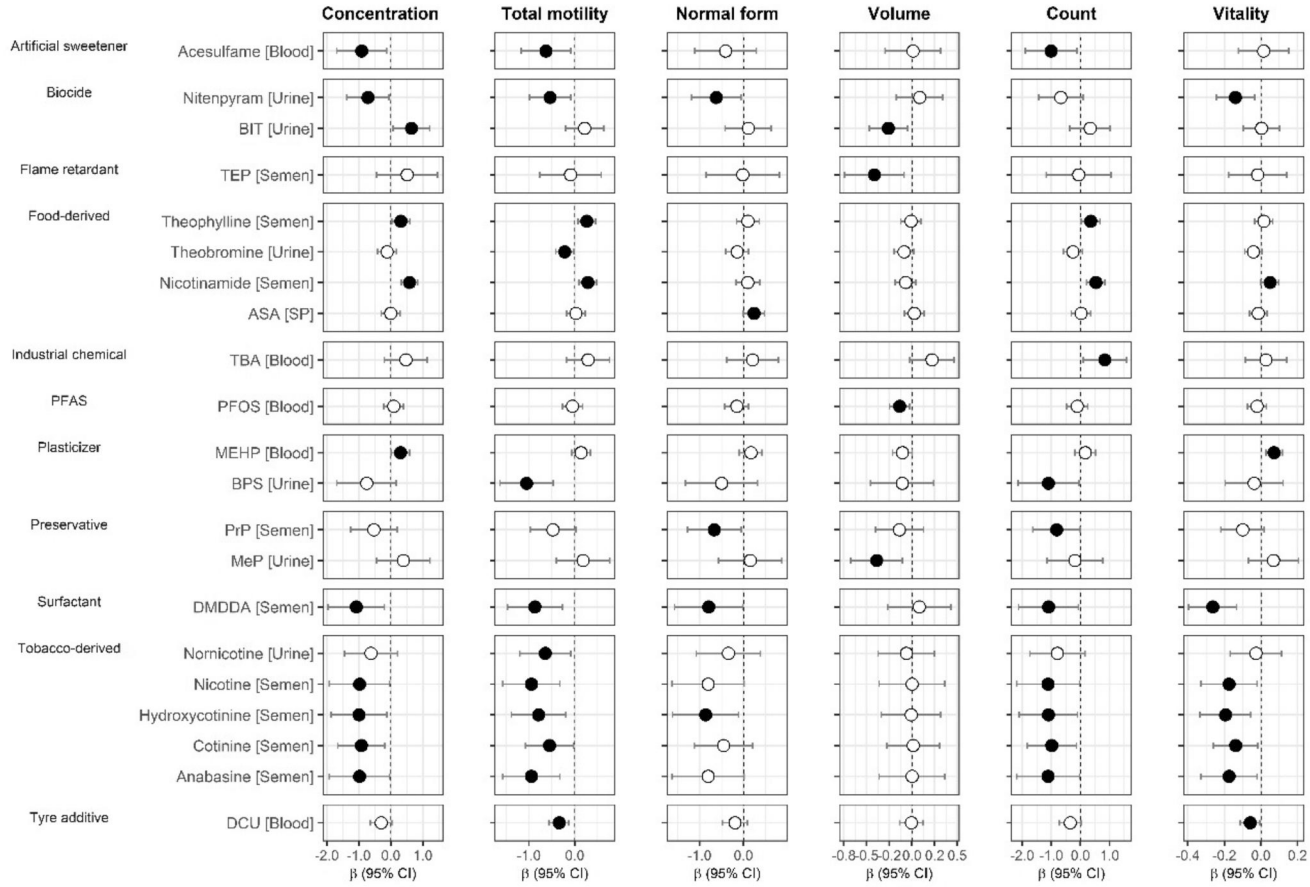

Figure 2. Forest plot summarizing the subset of associations between chemicals and semen quality parameters with at least one significant association across matrices. Specific associations for each chemical measured in seminal plasma (here referred as semen), blood plasma and urine can be found in Figures S7-S9. Chemicals with detection frequencies above 75% were modeled as continuous variables and those with detection frequencies (DF) between 25 and 75% as binary variables (see details of DF in Figure 1). The circles represent the regression coefficient (β) and the respective 95% confidence intervals (CI), filled with black color when the slope was statistically significant ($p < 0.05$). All models were adjusted for abstinence (days), age (years) and smoking (yes/no). Details of abbreviations can be found in Figure 1 and supplemental Table S6.

these results highlight the importance of considering both individual chemical toxicity and mixture interactions when assessing reproductive health impacts of environmental chemical exposures.

Discussion

In this study, we applied a novel wide-scope LC-HRMS based chemical profiling approach to characterize the occurrence of exogenous chemicals in urine, plasma and seminal plasma, and to explore their associations with semen quality. Our findings demonstrate that seminal plasma can harbor complex mixtures of exogenous chemicals from various environmental sources. Notably, up to 48 chemicals were detected in at least one sample of seminal plasma, and 5 chemicals were uniquely detected in this biological matrix. Statistical analyses revealed negative associations between several lesser-studied chemicals—such as DCU, propylparaben, bisphenol S or the biocide nitenpyram—and multiple semen parameters. In addition, our approach also confirmed known negative associations with PFAS and tobacco-related compounds, including nicotine and cotinine.

This investigation is among the first exposome-scale studies conducted on seminal plasma using matched urine and blood samples. Despite the interest of seminal plasma in chemical biomonitoring, most epidemiological studies on sperm quality have been conducted using more accessible matrices such as blood and urine, depending on the polarity of targeted chemicals, including pesticides,[24] bisphenol,[25] phthalates or organochlorines.[7,8] Our previous methodological article provides a thorough review of the existing studies analyzing and confirming the widespread presence of a wide range of organic chemicals in seminal plasma, including organophosphates, organochlorinated chemicals, PFAS, phthalic acid ester or volatile organic compounds.[17] Two recent studies have also profiled the chemical exposome of seminal plasma with wide-scope methods: one using a non-targeted LC-HRMS metabolomic approach,[26] and the other combining non-targeted and targeted gas chromatography coupled to HRMS strategies.[27] The first identified 30 exogenous chemicals in seminal plasma including phthalates, tobacco-related chemicals, phenols, and benzene metabolites, aligning well with our findings.[26] In the second case, the gas chromatography favored the identification of less polar chemicals, such as PCBs, dioxins and organochlorine pesticides, highlighting the complementary value of multi-platform analytical strategies in expanding exposome coverage.[27]

Given the limited number of studies employing matched matrices, the partitioning behavior of chemicals across the blood–testis barrier remains poorly understood. Our study contributes novel insights in this area. We observed generally positive correlations between matrices—specifically seminal plasma: blood

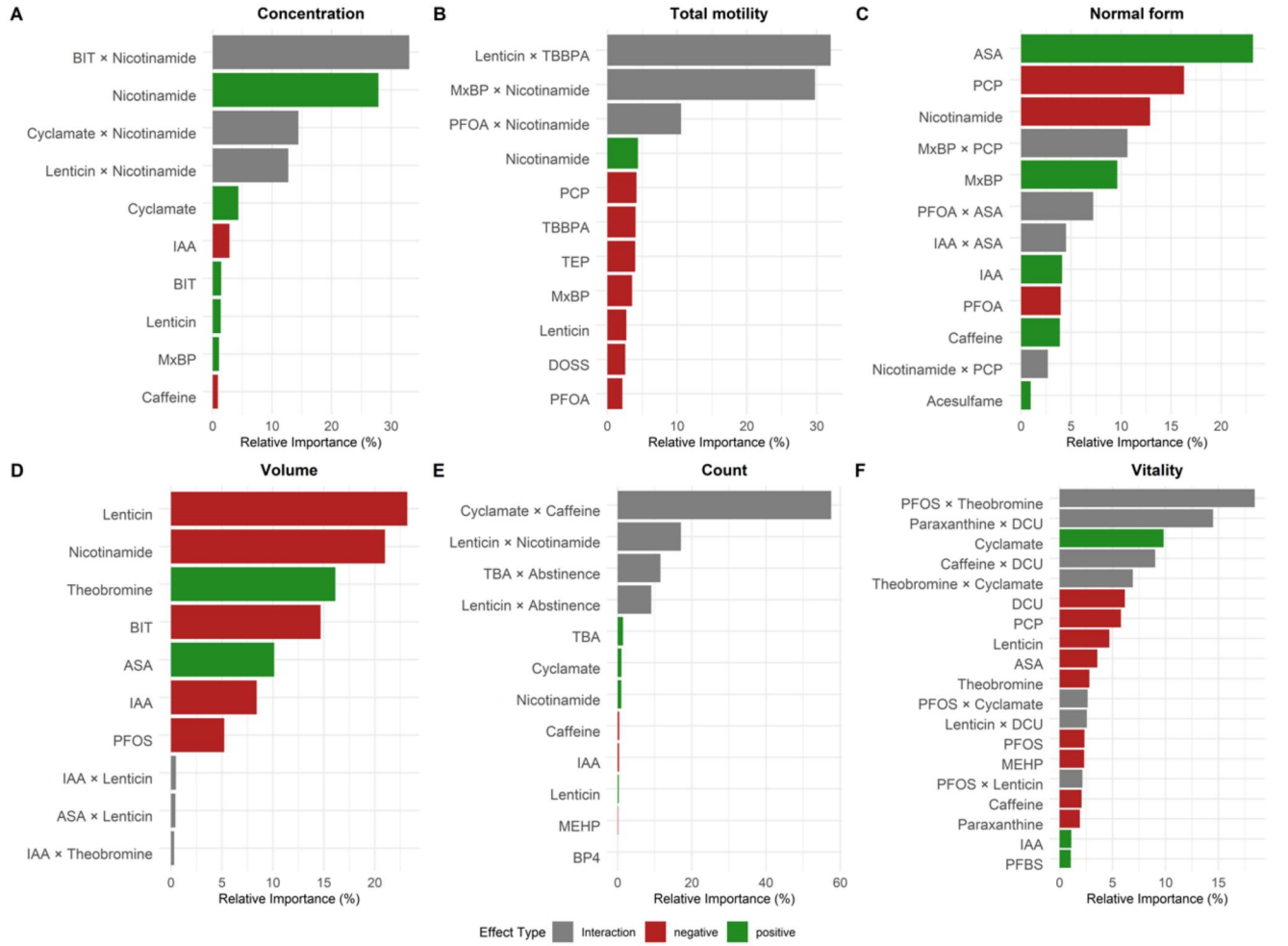

Figure 3. Bar plot depicting the relative contribution of variables and their interactions in the overall gradient boosting tree model for the sperm concentration (A), total motility (B), normal form (C), volume (D), count (E) and vitality (F). Green color indicates positive association and red color, negative association, calculated from individual partial dependencies. Marginal associations are depicted in the partial dependent plots for each variable and sperm quality model in supplemental materials (Figure S10-S12).

and seminal plasma: urine—for most chemicals. However, some chemicals showed null or even negative correlations, and up to five were detected exclusively in seminal plasma. Partition coefficient analysis revealed that most chemicals identified in this study tended to concentrate more in seminal plasma than in urine, while distribution relative to blood plasma was congener-specific. Previous studies with matched samples have reported consistent seminal plasma: blood PFAS ratios (approximately 0.4), with seminal plasma concentrations typically ranging from 0.02 to 0.2 ng/mL.[28,29] Although research is scarce, some chemicals—such as bisphenol A, which exhibits intermediate polarity, and the highly hydrophilic pesticide glyphosate—have been shown to accumulate more in seminal plasma than in urine or blood.[30,31] Noteworthy, direct measurement of PFAS in seminal plasma strengthened the negative associations with seminal parameters,[29] in agreement with our findings regarding tobacco-related chemicals or propyl paraben (supplemental Figure S13). Globally, these underscore the potential of seminal plasma as a valuable matrix for chemical biomonitoring, offering insight into the local testicular microenvironment where spermatogenesis occurs. Nevertheless, the complementarity of biological matrices may provide an asset to extend the coverage of chemical exposome landscape, balancing confounding bias and measurement error.[32]

Among the chemicals associated with semen quality, we highlight novel associations involving DCU in blood plasma, nitenpyram in urine, and propylparaben in semen. DCU, tire additive previously identified in serum[33] and environmental samples,[34] has limited toxicological data but is known to inhibit epoxide hydrolases—enzymes essential for lipid metabolism[35] essential for testosterone production.[36] To our knowledge, this is the first study reporting associations between urinary nitenpyram and reduced sperm concentration, motility, morphology, and vitality. Nitenpyram, a neonicotinoid insecticide used in agriculture and veterinary medicine, has been linked to metabolic alterations, testosterone levels, and oxidative stress responses in humans and animals.[37,38] As for parabens, previous studies offer mixed results: while some reported no associations,[39,40] others observed negative associations with sperm quality and testosterone levels.[41,42]. Our findings support the latter, identifying consistent negative associations for propylparaben in seminal plasma across multiple semen parameters. In contrast, the negative impacts of PFAS and tobacco-derived compounds (nicotine, cotinine) on semen quality are well documented and our results further validate these effects.[3,43,44] PFAS have been linked to reduced semen quality via mechanisms involving oxidative stress, apoptosis, and disruption of the blood–testis barrier.[43] We also observed positive associations between nicotinamide (vitamin

B3) in blood plasma and semen quality, aligning with its known role in cellular metabolism and antioxidant defense. Differential regulation of nicotinamide-related pathways has been reported in men with varying sperm quality.[45] Altogether, our findings support the framework proposed by Kortenkamp and colleagues,[9,10] using an adverse outcome network which highlights the combinatorial toxicity of environmental mixtures in male reproductive dysfunction through different modes or mechanisms of action.

Several limitations should be acknowledged. First, the relatively small sample size may limit statistical power, potentially missing modest associations. The size and mixed data structure (binary/continuous) also precluded the use of advanced multipollutant models (eg, BKMR). Moreover, the lack of correction for multiple testing may have led to false positives, though our main findings are consistent across matrices and endpoints. Single-spot sampling may introduce exposure misclassification, likely biasing results toward the null. Additionally, the cross-sectional design limits causal inference, underscoring the need for longitudinal studies. Residual confounding remains possible, particularly for chemicals with poorly understood biological pathways. On the analytical side, while our LC-HRMS method offers broad coverage, it may lack sensitivity for some low-abundance or low-polarity compounds.[15-17] Combining complementary techniques, such as GC-HRMS, is essential to improve coverage of the chemical exposome.

Our findings demonstrate that seminal plasma is susceptible to contamination by diverse environmental chemicals, including biocides, personal care products, pharmaceuticals, and industrial compounds. The wide-scope exposomic approach enabled the identification of DCU and nitenpyram as novel candidates for male reproductive toxicity, and reinforced existing evidence on the detrimental effects of parabens and tobacco-related compounds on semen quality. These results highlight the potential of seminal plasma as a matrix for chemical biomonitoring and emphasize the need for further studies to deepen our understanding of the seminal exposome and its role in male infertility.

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Author contributions

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Supplementary material

Supplementary material is available at *Exposome* online.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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