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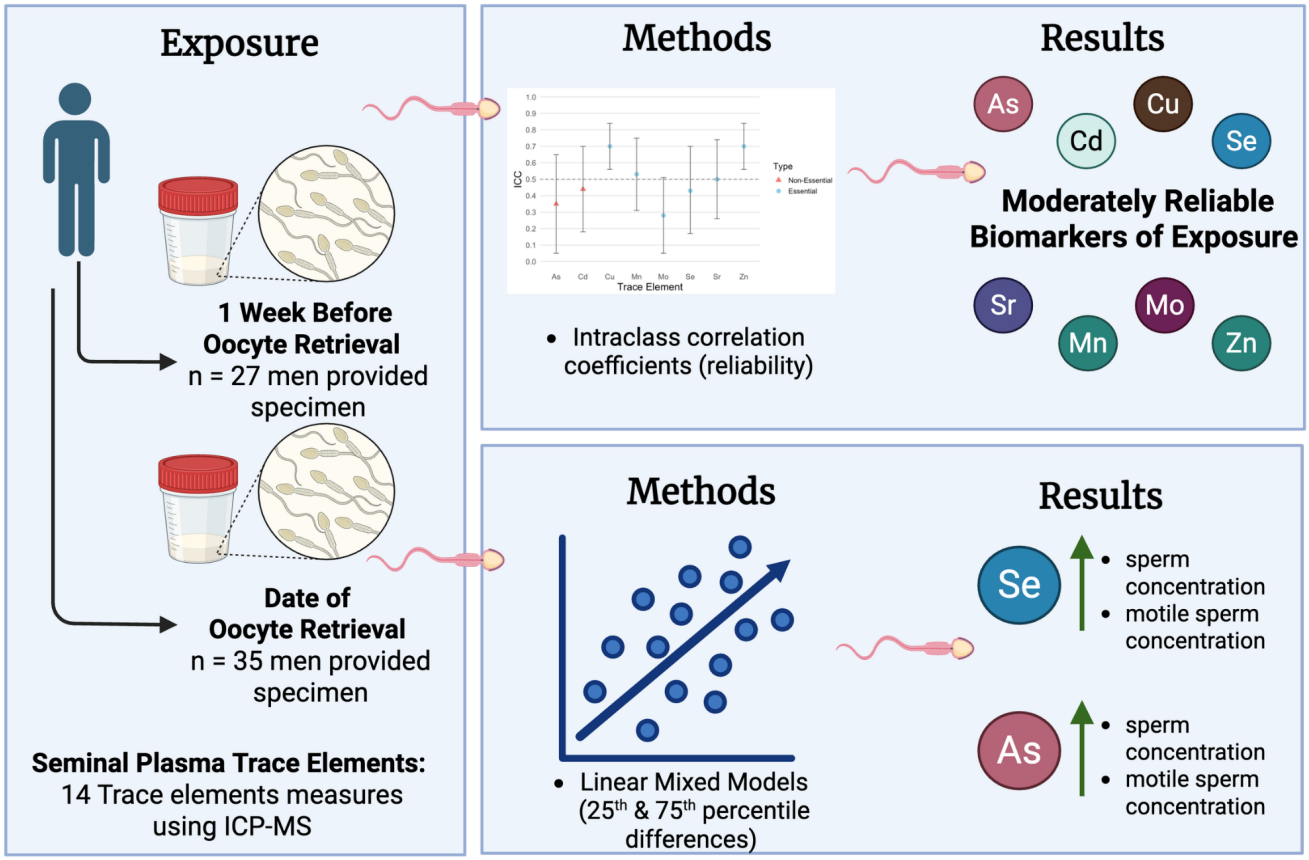
Graphical Abstract



Highlights:

- Seminal plasma trace element concentrations were a moderately reliable biomarker for As, Cd, Cu, Mn, Mo, Se, Sr, and Zn exposure.
- Between-person variability in trace element levels was greatest in Cu, Zn, and Mn.
- Greater seminal plasma As and Se were associated with higher sperm concentration.
- A mixture of high Cd, As, and Mo and low Cu and Se may predict poor sperm quality.

Seminal plasma trace elements: reliability as biomarkers and associations with sperm quality in male IVF patients

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Abstract: Studies of non-occupational exposure to trace elements (TE) and semen quality have had inconsistent results, and few studies have accounted for temporal variability or TE mixtures. To help address the data gap, we quantified associations between seminal plasma (SP) trace element concentrations and sperm quality measures among male partners of in vitro fertilization patients. We collected semen samples from 27 male partners an average of 8.6 days prior to the day of oocyte retrieval (time 1), and from 35 male partners on the day of oocyte retrieval (time 2). We determined concentrations of 14 TEs in SP using inductively coupled plasma-tandem mass spectrometry. We used linear mixed-effects regression to estimate associations between individual TEs, expressed as the change in sperm quality between the 25th and 75th percentiles of trace element distributions, adjusted for age, days between specimen collections, racial identity, recent seafood consumption, and cigarette smoking. We used principal component analysis (PCA) to estimate associations with TE mixtures at time 2. We found positive associations between SP Se and sperm concentration (%change = 47.75%; 95% CI: 4.20, 85.65) and motile sperm concentration (%change = 44.30%; 95% CI: 5.10, 88.75), and similar associations for SP As (%change = 30.50%; 95% CI: 6.85, 54.15 and %change = 25.15%, 95% CI: 12.55, 30.35, respectively). The PCA mixture of higher SP Cd, As, and Mo and lower Cu and Se suggests a pattern of poor sperm quality, though the associations were not statistically significant. Our findings underscore the importance of considering temporal variability of seminal plasma TE concentrations and suggest potentially important associations between exposure to essential TEs and semen quality which should be targeted for further investigation, including Se and As.

Keywords: Arsenic; *in vitro* fertilization (IVF); metals; selenium; seminal plasma; sperm quality; trace elements

1. Introduction

Infertility affects approximately 15% of couples in the United States, with male infertility contributing to roughly half of the cases [1,2]. Trace elements play important roles in male reproductive health and fertility [3]. Exposure to both essential trace elements, such as zinc (Zn) and selenium (Se) [4,5], and non-essential trace elements, such as cadmium (Cd) and arsenic (As), has been associated with sperm quality and male fecundity in experimental and epidemiologic studies [6,7]. Essential trace elements are required in small doses to maintain normal reproductive physiology, supporting spermatogenesis, oxidative stress regulation, and hormone function [6]. Non-essential trace elements, in contrast, serve no recognized biological function but may disrupt reproductive physiological processes, diminish sperm quality, and potentially impair fecundity [8]. These effects may be partly driven by greater oxidative stress, which can impair sperm and hormone functions [9]. For example, high doses of Cd interfere with testosterone production by inhibiting the release of hypothalamic gonadotropin-releasing hormone [10]. In an experimental study of mice, cadmium-induced oxidative stress impaired Leydig cell function, reduced testosterone synthesis, and disrupted spermatogenesis [11]. Higher Cd concentrations have also been associated with lower luteinizing hormone and follicle-stimulating hormone (FSH) levels in men, which may also interfere with spermatogenesis [12].

Despite evidence of reproductive toxicity from experimental and occupational studies at high levels, the associations between trace element exposures and male fecundity at low background exposure levels typically experienced

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by general populations remain understudied [13,14]. An epidemiologic study of couples using *in vitro* fertilization (IVF) found positive associations between Se levels in seminal plasma and clinical pregnancy [3]. Similarly, a randomized controlled trial found that increased concentrations of seminal plasma Se and Zn, supplemented with vitamin D, vitamin E, and serotonin, increased total sperm motility and reduced reactive oxygen species [15]. In contrast, higher seminal plasma concentrations of non-essential Cd and Pb were associated with lower sperm concentrations among men recruited from fertility clinics [16]. Despite these findings, research on background trace element exposure and male fecundity remains limited [17].

Most previous epidemiologic studies of male fecundity and fertility have focused on blood and urine trace element concentrations as biomarkers of exposure [14]. Yet, seminal plasma, the non-cellular component of semen, contains a rich profile of trace elements and may provide a more direct biomarker of exposure than indirect measures using blood and urine in studies of male fecundity [18]. A few human studies previously used trace element concentrations in seminal plasma as biomarkers of exposure, including heavy metals [19]. However, most trace elements have short *in vivo* half-lives and concentrations are likely to vary over short time intervals within subject, which may undermine their suitability for epidemiologic studies [20]. No studies, to our knowledge, have assessed the within-person and between-person variabilities and reliability of seminal plasma trace elements as biomarkers of exposure.

Though trace element concentrations in seminal plasma have substantial potential for use as a biomarker of exposure, it remains underexplored in human studies [14,18]. To address this data gap, our objectives were (1) to characterize the variability and reliability of trace element concentrations in seminal plasma as biomarkers of exposure in the male partners of IVF patients, and (2) to estimate associations between seminal plasma trace element concentrations and sperm quality measures. The results of this study may guide the future use of trace element concentrations in seminal plasma as biomarkers of exposure and generate testable hypotheses to inform the design of a larger confirmatory study that will help to guide practitioners to improve male fecundity.

2. Methods

2.1. Study participants

Participant enrollment and the analysis of seminal plasma trace elements have been detailed previously [21–24]. Briefly, we recruited 58 female patients undergoing IVF treatment at the University of California at San Francisco (UCSF), as well as 37 male partners, to the Study of Metals and Assisted Reproductive Technologies (SMART) between October 2015 and June 2017. We collected seminal plasma from male partners at two time points (Supplementary Figure S1). Participants were instructed to masturbate into a sterile specimen collection cup, without lubricant, after at least two days of abstinence. At time 1, semen specimens were collected at home (n = 27), refrigerated, and delivered to UCSF for processing the next morning. Time 2 specimen collection was on-site at UCSF on the day of oocyte retrieval after at least two days of abstinence (n = 35), according to the standard clinical protocol. Fresh semen samples were transferred to plastic tubes and centrifuged using a density gradient silica colloid preparation at a 1:1 ratio to isolate sperm. The seminal plasma layer, which is normally discarded as waste after sperm extraction, was then divided into 0.5 mL cryovials and immediately frozen at −80 °C for analysis. All laboratory disposables were either acid-washed and sealed to prevent exogenous contamination before use or screened for systematic contamination by the analyzing laboratory using a protocol consistent with that used by the CDC for the National Health and Nutrition Examination Survey [25]. Participants completed a questionnaire on dietary and lifestyle factors on the day of oocyte retrieval.

All participants completed informed consent prior to enrollment, and the study protocol was approved by the UCSF Committee on Human Research, the University at Albany, State University of New York, and the Wadsworth Center, New York State Department of Health.

2.2. Analysis of sperm quality outcomes

Semen analyses were conducted at the UCSF Andrology Laboratory in accordance with the World Health Organization (WHO) criteria [26]. Semen volume was measured by weight using pre-weighed sterile collection containers; post-collection weights were recorded, and the difference was used to calculate the ejaculate volume, with daily quality control performed using 1 g and 5 g reference weights. Total sperm count (millions) was calculated as sperm concentration × semen volume. Total motile sperm count (millions) was calculated as total sperm count x (percent motile/100). Sperm concentration and motility were measured manually using a Leja Standard Count two-chamber slide (Conception Technologies, MC20-2) under a standard microscope at 400× power (Nikon, Inc. Melville, NY, USA). Total sperm concentration (millions/mL) was calculated using manufacturer calibration factors to account for the known chamber volume and magnification (20× objective = factor of 20; 40× objective = factor of 80). Motile sperm concentration (millions/mL) was calculated as (motile sperm count/10) × 8 (correction factor). Percent motility (%) was calculated as total sperm concentration/total motile sperm concentration × 100.

2.3. Analysis of seminal plasma trace elements

Procedures for the analysis of trace elements in seminal plasma were previously described in detail [24]. Seminal plasma specimens were shipped from UCSF to the Wadsworth Center, New York State Department of Health (Albany, NY, USA) for analyses using a method developed for this project and validated for the analysis of 14 elements including: As, barium (Ba), Cd, chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), molybdenum (Mo), Se, strontium (Sr), uranium (U), vanadium (V), and Zn. The specimens were

deproteinized with concentrated nitric acid and diluted (1 + 24) for analyses using an Agilent 8900 Inductively Coupled Plasma Tandem Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA). We analyzed 19 seminal plasma samples in duplicate. There were 9 participants who provided a specimen at time 1 but did not at time 2. An additional 16 participants provided specimen at time 2. The limits of detection (LOD) were determined as three times the standard deviation of seven independent analyses of a seminal plasma-based quality control sample, following ISO/IUPAC guidelines for single-laboratory method validation, as follows [24,27]: As: 0.3 µg/L, Ba: 0.7 µg/L, Cd: 0.02 µg/L, Cr: 1.2 µg/L, Co: 0.05 µg/L, Cu: 11 µg/L, Pb: 0.08 µg/L, Mn: 0.5 µg/L, Mo: 0.9 µg/L, Se: 14 µg/L, Sr: 2.5 µg/L, U: 0.03 µg/L, V: 0.5 µg/L, and Zn: 11 mg/L. The analysis of clinical reagent blanks suggested exogenous contamination from Ba, Cr, Co, Pb, U, and V; these elements were excluded from further analysis.

2.4. Statistical analysis

We characterized the distributions of seminal plasma trace elements and sperm quality outcomes. We square-root transformed sperm quality outcome measurements to approximate a normal distribution. We used Spearman correlation coefficients to estimate pairwise correlations between log-transformed seminal plasma trace element concentrations.

2.4.1. Seminal plasma trace element variability and reliability

We used linear mixed-effects regression models to estimate variance components for seminal plasma trace element measurements. We quantified the proportion of variability due to sources between subjects, within subjects, and due to analytic factors. We estimated the intraclass correlation coefficients (ICC), which describe the correlation between any two seminal plasma trace element measurements from the same subject, in which a higher value corresponds to greater reliability for use as biomarkers of exposure in observational studies [28]. We also calculated the number of specimens needed to estimate subject-specific mean values (k), as $k = [R(1 - ICC)] / [ICC(1 - R)]$, where R represents the desired reliability of the subject-specific mean [29].

2.4.2. Individual seminal plasma trace element predictors of sperm quality outcomes

We estimated associations between individual log-transformed seminal trace element measurements at time 1 and time 2 as predictors of square-root transformed sperm quality measurements as outcomes, using separate linear mixed-effects linear regression models for each element. Because the dose-response associations were non-linear, we expressed effect estimates as the expected percentile difference in sperm quality outcome value for a difference between the 25th and 75th percentiles of the seminal plasma trace element concentrations. We adjusted models for age (years), abstinence (days without ejaculation), racial identity (Asian *vs.* Other), cigarette smoking (≥ 100 cigarettes in lifetime *vs.* < 100 cigarettes in lifetime), and recent seafood consumption (> 1 lb. in the prior week *vs.* < 1 lb. in prior week). We identified these confounding variables as factors that predicted both exposure to trace elements and semen quality, and did not fall within the causal pathway in previous literature [30–34]. We then used a directed acyclic graph to retain a minimal set of variables to prevent confounding (Supplementary Figure S2) [35]. Smoking status was missing for n = 24 (~ 69%) and was imputed as "non-smoker". In a sensitivity analysis, we imputed missing values as "smoker" in adjusted models under an extreme-case assumption for a known confounder [36].

2.4.3. Multiple seminal plasma trace element predictors of sperm quality outcomes

We used principal component analysis (PCA) to summarize the variability of multiple seminal plasma trace element levels. PCA is an unsupervised dimension reduction technique that transforms multiple correlated variables into fewer orthogonal, or uncorrelated, components [37]. To ensure stable results, we limited the PCA analysis to time 2 semen collections (n = 35) and implemented a varimax rotation. We retained two principal components to summarize the eight seminal plasma trace elements based on eigenvalues > 1 (Supplementary Figure S3). We then used the two components as predictors of sperm quality outcomes in general linear regression models, adjusting for covariates, to quantify the associations between the mixture of multiple seminal plasma trace elements and sperm quality at time 2. In a sensitivity analysis, we imputed n = 24 with missing smoking information as "smoker" in adjusted models.

We defined $p < 0.10$ as statistically significant for a two-tailed hypothesis test to maximize the detection of hypotheses for confirmation in a future study as implemented by prior exploratory biomarker studies, and consistent with the hypothesis-generating nature of this study [38]. R statistical software version 4.4.3 (R Foundation for Statistical Computing, Vienna, Austria) was used for the analysis.

3. Results

3.1. Participant demographic and clinical factors

Demographic and clinical characteristics of the study participants are shown in Table 1. The mean age of participants was 39.4 years, with a range of 31–49 years. Nearly half (45.7%) of the study population identified as Asian race, and 54.3% identified as another race (primarily White). Most (60%) participants reported consumption of > 1 lb. of seafood in the prior week. More than 14% of participants smoked at least 100 cigarettes in their lifetime (*i.e.*, "ever" smokers).

Sperm quality measures are also shown in Table 1. The average total sperm count was 232.80 million, with a range of 31–1052.1 million, and the average total sperm concentration was 79.2 million/mL, with a range of 0.0–373.0 million/mL. The average motile sperm concentration was 46.4 million/mL, with a range of 0.0–238.7 million/mL, and the percent motile sperm was 46.8%, with a range of 0%–87%. We found one participant who presented with azoospermia.

Table 1. Demographic characteristics and sperm quality outcomes of male partners of IVF patients (n = 35).

Characteristic	n	Mean ± SD or percent	Minimum	25th percentile	Median	75th percentile	Maximum
Age (years)	35	39.4 ± 4.3	31	36	40	42	49
Ever Cigarette Smoker [a]							
Yes	5	> 14%	-	-	-	-	-
No	30	85.7%	-	-	-	-	-
Racial Identity							
Asian	16	45.7%	-	-	-	-	-
Other	19	54.3%	-	-	-	-	-
Recent Seafood Consumption [b]							
Yes	21	60.0%	-	-	-	-	-
No	14	40.0%	-	-	-	-	-
Time Between Specimen Collections (days)	35	8.6 ± 3.4	4	5	8	12	15
Total Sperm Count (millions)	35	232.8 ± 222.4	31.0	81.0	176.8	336.0	1052.1
Sperm Concentration (millions/mL)	35	79.2 ± 75.3	0.0	29.4	58.0	1052.0	373.0
Motile Sperm Concentration (millions/mL)	35	46.4 ± 56.8	0.0	8.0	29.9	57.8	238.7
Percent Motile Sperm (%)	35	46.8 ± 19.9	0.0	34.0	45.0	46.8	87.0

Abbreviations: n, sample size, SD, standard deviation.
NOTE: [a] Smoked at least 100 cigarettes in lifetime; [b] Consumed > 1 lb. seafood in the past week.

3.2. Seminal plasma trace element concentrations as biomarkers of exposure

3.2.1. Seminal plasma trace element concentration variability and reliability

The distributions of non-essential and essential elements measured in seminal plasma at time 1 and time 2 are described in Table 2. Most seminal plasma elements were measured above the LOD at times 1 and 2. Median seminal plasma Zn concentration was lower at time 1 than at time 2, while the concentrations of other seminal plasma trace elements were similar at times 1 and 2. The pairwise correlations between seminal plasma trace element concentrations measured at time 1 and time 2 are shown in Supplementary Figure S4. Seminal plasma concentrations of essential elements Cu and Zn were significant positive correlations at time 1 and time 2. The strength of the correlations between trace elements varied between time 1 and time 2. For example, correlations between Mo and Se were generally stronger at time 2 than at time 1, suggesting variability in the relationships among seminal plasma trace elements across time points.

Table 2. Distributions of seminal plasma trace element concentrations (µg/L) among male partners of IVF patients at (A) time 1 (n = 27), and (B) time 2 (n = 35).

Element	LOD	% > LOD	Mean ± SD	Minimum	25th percentile	Median	75th percentile	Maximum
(A) Time 1								
As	0.3	82.1	1.96 ± 1.81	0.13	0.68	1.54	2.50	7.61
Cd	0	96.4	0.19 ± 0.14	0.03	0.10	0.14	0.23	0.56
Cu	11	100	88.02 ± 35.11	43.28	52.14	84.65	117.33	163.93
Mn	0.5	100	9.33 ± 4.81	2.10	6.33	8.90	11.29	24.15
Mo	0.9	100	2.92 ± 1.24	1.19	1.80	2.92	3.70	5.97
Se	13.9	100	76.52 ± 32.70	22.90	56.42	74.60	85.82	151.80
Sr	2.5	100	92.53 ± 41.41	46.92	63.01	80.40	108.38	223.13
Zn	11.4	100	141.71 ± 85.44	35.74	72.83	112.05	197.45	320.93
(B) Time 2								
As	0.3	84.6	1.50 ± 1.51	0.19	0.47	0.84	2.01	6.98
Cd	0	92.3	0.18 ± 0.14	0.04	0.09	0.13	0.19	0.60
Cu	11	100	80.98 ± 35.40	20.71	52.48	80.52	102.77	200.48
Mn	0.5	100	9.35 ± 4.33	3.17	5.69	9.35	9.16	21.62
Mo	0.9	100	3.38 ± 1.99	1.11	2.25	2.92	3.87	12.33
Se	13.9	100	69.20 ± 24.11	23.73	51.09	67.31	88.05	110.28
Sr	2.5	100	95.76 ± 51.67	44.94	66.09	84.00	99.51	317.36
Zn	11.4	94.9	122.82 ± 55.14	7.62	90.36	122.22	154.75	237.85

Abbreviations: LOD, limit of detection; SD, standard deviation.

As shown in Table 3, measurement variability between males (*i.e.*, between participants) was greatest for Cu (70.3%), Zn (69.6%), Mn (53.3%), and Sr (50.0%). Measurement variability within men (*i.e.*, between sample collections)

was greatest for Mo (67.9%), Se (52.6%), and Sr (48.7%). As shown in Figure 1, ICCs, which describe the correlation between repeated specimens within each participant, were 0.70 (95% CI: 0.54, 0.82) for Zn, 0.70 (95% CI: 0.56, 0.84) for Cu, 0.53 (95% CI: 0.31, 75) for Mn, 0.43 (95% CI: 0.17, 0.70) for Se, 0.50 (95% CI: 0.26, 0.74) for Sr, 0.28 (95% CI: 0.05, 0.51) for Mo, 0.35 (95% CI: 0.05, 0.65) for As, and 0.44 (95% CI: 0.18, 0.70) for Cd. We calculated that two specimens are required to estimate mean seminal plasma Cu concentration with ≤ 15% error, while 3–5 specimens are needed for Se (k = 3), Zn (k = 3), Mn (k = 4), Sr (k = 4), and Mo (k = 5), and other trace elements required more.

Table 3. Characteristics of variability of seminal plasma trace element concentrations among male partners of IVF patients (n = 35).

Element	GM	GSD	GCV%	σ^2_{total}	$\%\sigma^2_{between}$	$\%\sigma^2_{within}$	$\%\sigma^2_{analytical}$	$m_{5\%}$	$m_{10\%}$	$m_{15\%}$	$m_{20\%}$
As	1.70	1.61	94.70	3.16	35.01	43.26	21.73	25	12	8	6
Cd	0.18	0.14	77.80	0.03	44.44	11.11	44.44	24	12	8	6
Cu	84.50	35.26	41.70	1179.88	70.29	28.16	1.55	7	4	2	2
Mn	9.11	4.59	50.40	19.98	53.30	37.60	9.20	12	6	4	3
Mo	3.15	1.59	50.50	2.77	28.30	67.87	3.83	14	7	5	4
Se	71.41	29.59	41.40	880.25	43.03	52.61	4.36	9	5	3	2
Sr	87.53	46.59	53.20	2079.25	50.00	48.69	1.31	13	7	4	3
Zn	134.44	73.15	54.40	5201.10	69.62	29.40	0.98	9	5	3	2

Abbreviations: GM, geometric mean of time 1 and time 2 samples; GSD, geometric standard deviation of time 1 and time 2 samples; %GCV, geometric coefficient of variation describing the between-subject variability of time 1 and time 2 samples; σ^2_{total}, total variability; $\%\sigma^2_{bewteen}$, relative variability between men; σ^2_{within}, relative variability between Time 1 and Time 2 specimen retrievals; $\%\sigma^2_{analytical}$, relative analytical variability; $m_{5\%;}$ $m_{10\%}$; $m_{15\%}$; $m_{20\%}$, minimum number of samples needed to estimate the mean value with 5%, 10%, 15%, and 20% error, respectively.

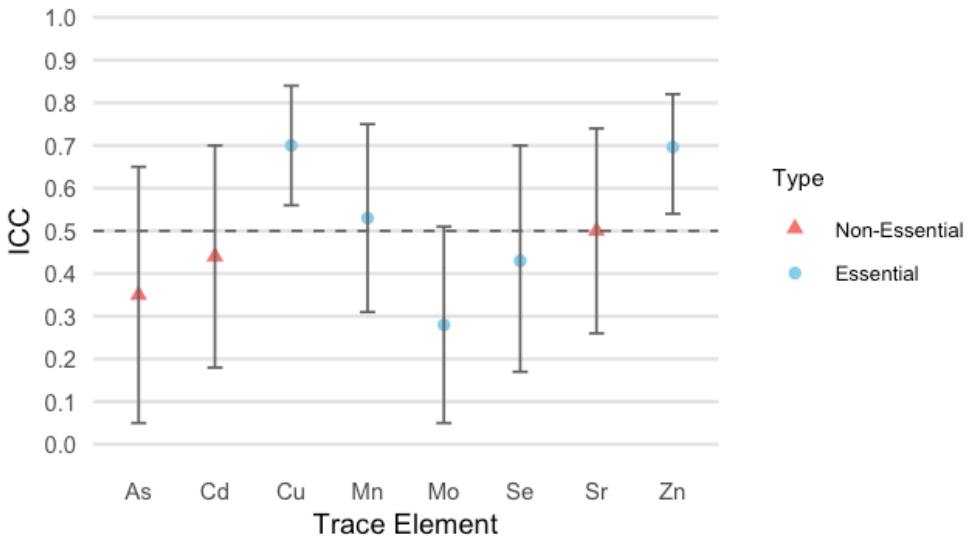

Figure 1. Intraclass correlation coefficients with 95% confidence intervals for essential and non-essential trace elements in seminal plasma samples among male partners of IVF patients (n = 35). Abbreviations: ICC, Intraclass correlation coefficient. Note: Reference line at ICC = 0.5 indicates threshold for moderate reliability.

3.2.2. Individual seminal plasma trace element predictors of sperm quality outcomes

Table 4 shows the covariate-adjusted associations between seminal plasma trace element concentrations and sperm quality outcomes across times 1 and 2 as percent changes per IQR increase in trace element concentration. Greater plasma As and Se were associated with higher sperm concentration (% change: 30.50% per IQR increase, 95% CI: 6.85, 54.15; % change: 47.75%, 95% CI: 4.20, 85.65, respectively), and motile sperm concentration (% change: 25.15%, 95% CI: 12.55, 30.35; % change: 44.30%, 95% CI: 5.10, 88.75, respectively) when adjusted for age, time between specimens, racial identity, and recent seafood consumption, with As additionally adjusted for cigarette smoking. The unadjusted results were similar (Supplementary Table S1). Our results were similar when we imputed missing smoker data as smokers in the As and Cd models (Supplementary Table S2), and when we excluded n = 1 azoospermic participant (data not shown).

Table 4. Adjusted associations between individual seminal plasma trace elements and sperm quality outcomes among male partners of IVF patients (n = 35).

Elements	Total Sperm Count		Sperm Concentration		Motile Concentration		Percent Motility	
	% Change	95% CI	% Change	95% CI	% Change	95% CI	% Change	95% CI
As [a, b]	18.20	−19.75, 37.25	30.50 *	6.85, 54.15	25.15 *	12.55, 30.35	25.45	−1.15, 30.00
Cd [a, b]	11.60	−15.25, 32.45	16.10	−3.25, 19.80	−9.25	−14.05, 3.75	17.10	−14.55, 10.85
Cu [a]	27.65	−6.45, 47.35	20.35	−2.10, 24.05	12.10	−1.80, 14.45	15.05	−0.65, 61.30
Mn [a]	15.30	−10.25, 42.60	28.45	−5.60, 39.12	23.75	−1.85, 14.60	44.18	−8.05, 57.25
Mo [a]	−8.75	−70.20, −58.55	−6.55	−25.10, 4.75	−18.10	−27.15, 33.45	−4.25	−38.25, 33.15
Se [a]	42.10	−3.05, 48.85	47.75 *	4.20, 85.65	44.30 *	5.10, 88.75	63.40	−3.05, 87.80
Sr [a]	4.90	−2.25, −10.05	1.75	−3.15, 5.85	4.55	−38.60, 70.40	8.05	−58.10, 69.80
Zn [a]	1.85	−16.10, 19.90	−8.45	−16.25, 5.35	−5.65	−19.25, 4.15	−31.45	−36.20, 3.80

NOTE: Effect estimate for the difference between the 25th percentile and 75th percentile of seminal plasma trace element concentrations (μg/L); [a] adjusted for age (years), days between specimen collections, racial identity (Asian *vs.* non-Asian), and recent seafood consumption (≥ *vs.* < 1 lb. in the past week); [b] also adjusted for cigarette smoking (ever *vs.* never); * P-value < 0.10.

3.2.3. Multiple seminal plasma trace element predictors of sperm quality outcomes

Based on 95% confidence intervals that overlapped ICC = 0.5, we retained seminal plasma As, Cd, Cu, Mn, Mo, Se, Sr, and Zn as potential predictors of sperm quality outcomes at time 2 in a mixture assessment using PCA. As shown in the factor loadings in Figure 2, Component 1 (RC1) reflected higher non-essential elements Cd and As, higher Mo, as well as lower Se and Cu. Component 2 (RC2) reflected higher seminal plasma Zn, Se, Mn, and Cu, essential elements. We found associations between greater non-essential trace element concentrations (RC1) and lower total sperm count and motile sperm concentration in the covariate-adjusted models (% change: −1.48, 95% CI: −4.33, 1.43; % change: −2.17, 95% CI: −3.95, 2.17, respectively) (Figure 3 and Supplementary Table S3), albeit without statistical significance. Our results were consistent in a sensitivity analysis when we imputed missing smoker data as smokers (Supplementary Figure S5).

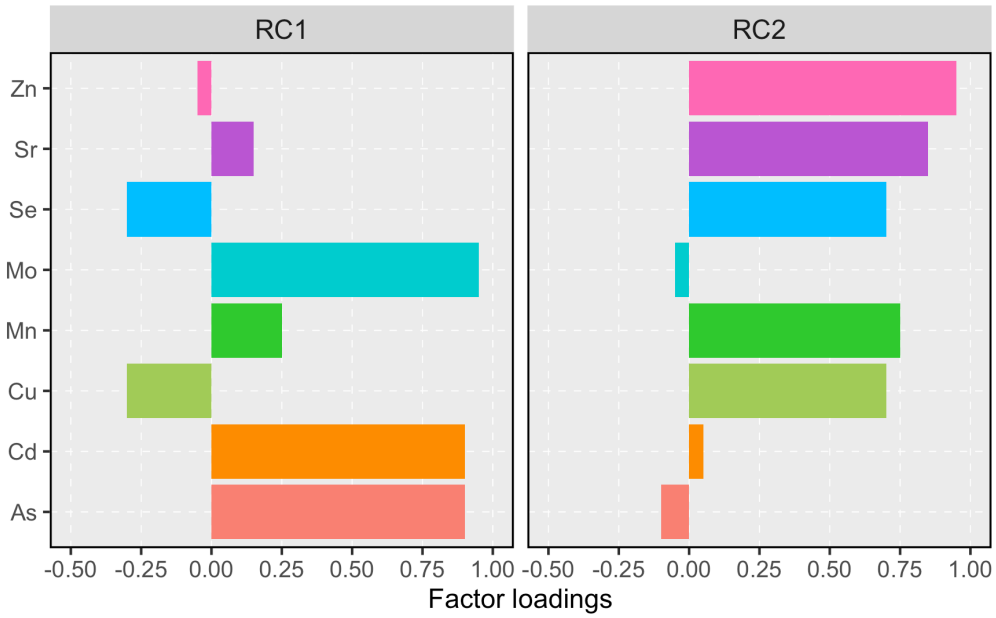

Figure 2. Factor loadings for varimax rotated principal components describing time 2 seminal plasma of trace elements among male partners of IVF patients (n = 35). Abbreviations: RC1, First rotated (principal) component, representing higher toxic element concentrations and lower essential element concentrations; RC2, Second rotated (principal) component, representing higher essential element concentrations.

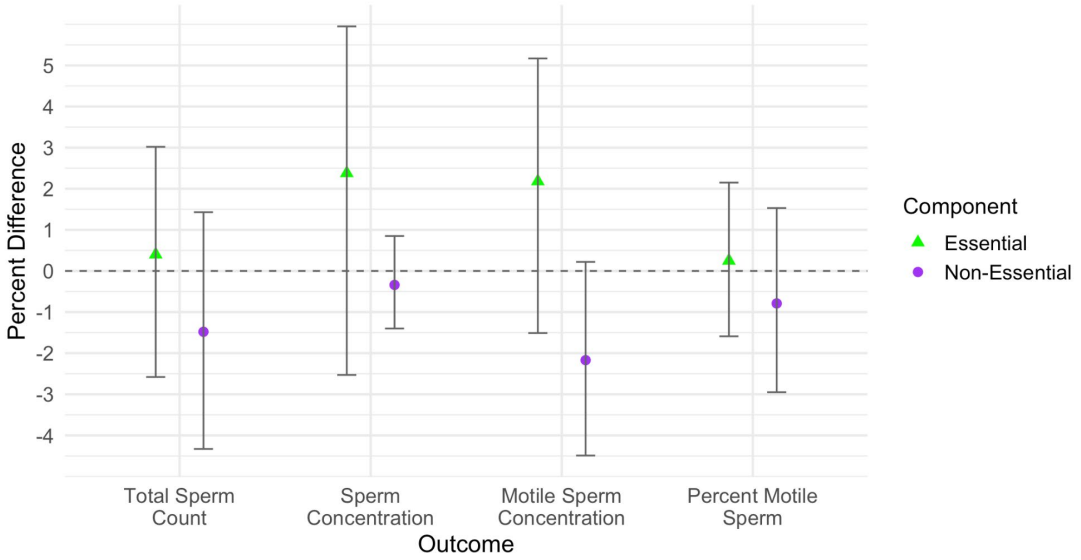

Figure 3. Adjusted associations between multiple seminal plasma trace elements and sperm quality outcomes among male partners of IVF patients at time 2 (n = 35). NOTE: Relative difference in sperm quality outcome for the difference between the 25th percentile and 75th percentile of seminal plasma trace element concentrations (µg/L), also adjusted for age (years), days abstinent before providing specimen, race (Asian *vs.* Other), seafood consumption (> 1 lb. in the past week), and cigarette smoking (ever *vs.* never).

4. Conclusion

4.1. Primary findings

In this hypothesis-generating study, we estimated associations between seminal plasma trace elements and sperm quality outcomes among male partners of IVF patients with repeated seminal plasma collections. We found that As, Cd, Cu, Se, Sr, Mn, Mo, and Zn concentrations in seminal plasma were moderately reliable biomarkers of exposure over a short time interval, indicated by 95% ICC confidence intervals that included ICC > 0.5 [39]. We found that 2 to 5 repeated specimen collections were required for a reliable estimation of the mean concentrations of most trace elements in seminal plasma. As individual predictors of sperm quality outcomes, greater seminal plasma As and Se concentrations were associated with higher total and motile sperm concentrations. Our results also suggested that higher levels of seminal plasma As, Cd, and Mo in conjunction with lower seminal plasma Cu and Se levels were associated with poorer sperm quality outcomes, adjusted for other seminal plasma trace elements and covariates. These findings suggest that exposure to seminal plasma trace elements, particularly As, Se, Cd, Mo, and Cu may be important predictors of sperm quality, highlighting the importance of repeated semen specimen collections for accurate measurement.

4.2. Seminal plasma trace elements and sperm quality outcomes

Previous studies have reported that greater blood Se concentrations were associated with better sperm quality and fertility outcomes in experimental and observational studies [40]. Higher levels of Se exposure have been associated with increased sperm production, antioxidant defense, and DNA stability in animal models [41]. Despite a moderate correlation between seminal plasma Se concentrations measured at T1 and T2 in our study (*i.e.*, ICC = 0.43; 95% CI: 0.26, 0.74), we found consistent positive associations between seminal Se and sperm quality outcomes. In alignment with our results, a cross-sectional investigation of 113 male partners of female IVF patients in Iran reported significant correlations between greater seminal plasma Se (mean = 126.44 ng/mL) and normal semen quality [42]. Similarly, a cross-sectional study of 394 men from a Chinese infertility clinic found that higher urinary Se concentration was associated with lesser odds of sperm concentration < 20 million/mL (odds ratio (OR) = 0.30, 95% CI: 0.09, 0.90) and sperm motility < 50% motile (OR = 0.23, 95% CI: 0.06, 0.86), based on reference levels set by the WHO, adjusted for other urinary trace elements and income [32]. Se is a key component of glutathione peroxidase and other selenoproteins, which protect spermatozoa from oxidative damage [40,43]. Our prospective results using seminal plasma Se further support a protective role for Se on sperm quality, though we found lower Se concentrations than prior studies.

Cu and Zn are essential elements that have also been associated with sperm health in human studies [6]. A cross-sectional study of 42 Turkish men with abnormal sperm quality and 10 control subjects with seminal plasma Zn and Cu concentrations similar to our study, reported positive correlations between Zn (median = 140.80 mg/L) and sperm count ($r = 0.36$, $p < 0.01$) and total motility ($r = 0.62$, $p < 0.01$); they also reported positive correlations between Cu with sperm count ($r = 0.36$, $p < 0.01$) and total motility ($r = 0.62$, $p < 0.01$) [44]. A cross-sectional study of 72 Iranian men also reported a positive association between seminal plasma

Zn concentration and measures of sperm quality [45], and another study of 746 Chinese men found a positive correlation between urinary Zn and sperm concentration [46]. Conversely, a study of 413 U.S. men with repeated semen collections found a negative association between blood Cu and total sperm count (mean difference = −1.30, 95% CI: 2.47, −0.14), adjusted for abstinence time, body mass index, and education [30]. We did not find associations between seminal plasma Cu and Zn with semen quality outcomes in our study. The discrepant results may be due in part to the limited number of participants in our study, differences in exposure levels to trace elements, and sociodemographic and lifestyle differences across the study populations. Still, we found a trend towards an association between lower seminal plasma Cu and poorer semen quality, in conjunction with higher Cd, As, Mo, and lower Se, although the effect estimates were imprecise. Based on our reliability analyses, incorporating at least 2 to 3 semen collections is necessary to more clearly define the associations between Cu and Zn exposure and semen quality. A larger prospective study is needed to test the hypothesis that coexposure to a seminal plasma mixture of higher Cd and Mo, with lower Cu and Se, as potential biomarkers of lower fecundity.

Cd and As are non-essential elements associated with adverse reproductive health outcomes, including impaired sperm function [14]. A cross-sectional study of 34 infertile males in Poland reported that high seminal plasma Cd levels were negatively correlated with sperm count and motility ($p < 0.01$) [47]. An Italian cross-sectional study reported that greater seminal plasma Cd was associated with low sperm count (OR = 4.48, 95% CI 0.25, 80) and low progressive sperm motility (OR = 3.45, 95% CI 0.77, 16), although imprecise and without statistical significance [31]. The aforementioned study from a Chinese infertility clinic reported an inverse relationship between urinary Cd and total sperm motility [32]. From the aforementioned Turkish study, blood Cd concentrations were associated with lesser sperm motility ($r = -0.28$, $p = 0.040$); however, the negative association was stronger for seminal plasma Cd ($r = -0.38$, $p = 0.005$), suggesting that trace element concentrations of seminal plasma had greater sensitivity than blood as a biomarker of exposure [44]. While we did not detect a significant association between Cd and sperm quality, PCA revealed a suggestive association between higher seminal plasma Cd, As, Mo, and lower Cu and Se with poorer sperm quality. However, when considered alone, we found that greater seminal plasma As was associated with higher motile sperm concentration. This unexpected result may have been a chance finding related to the small sample size, residual confounding by seafood consumption, or the non-speciated measurement of As. Greater seafood consumption is associated with improved sperm quality and organic As species are found in high concentrations in seafood [48,49]. We measured total and As in our study and our crude measure of weekly seafood consumption was imprecise. For a more definitive result, a future study should incorporate speciated analysis of As, to distinguish innocuous organic As species from non-essential inorganic As species, and collect more detailed seafood consumption data. Though our results suggest that Cd and As concentrations in seminal plasma may be a moderately reliable biomarker of exposure, the high levels of variability in seminal plasma underscore a need for repeated seminal plasma measurements to reduce exposure measurement error.

4.3. Biological mechanisms

Trace elements play a critical role in biological processes that influence sperm quality. The positive associations between seminal plasma Se and sperm quality outcomes in this study align with the roles of Se in mitigating oxidative stress, a major contributor to sperm dysfunction [50]. In our mixtures analysis, we found that co-exposure to a mixture of higher non-essential elements (Cd, As, and Mo) and lower essential elements (Se and Cu) may have negative implications for sperm quality. Non-essential trace elements like Cd may impair sperm quality by inducing oxidative stress, disrupting essential trace element homeostasis, and interfering with endocrine regulation [51]. Higher concentrations of Cd are known to damage mitochondrial function, specifically through the electron transport chain, which may increase the production of reactive oxygen species and inhibit antioxidant enzymes, which increase oxidative damage to sperm membranes and DNA [52,53]. Cd can also alter hypothalamic-pituitary-gonadal axis function, affecting gonadotropin secretion, which is essential for testosterone production and spermatogenesis [54]. While we did not detect a statistically significant association, we found suggestive evidence of associations between non-essential elements, including seminal plasma Cd, and sperm quality, which may be consistent with this effect. Poor sperm quality outcomes, may be attributable to sperm membrane damage from oxidative stress and reduced antioxidant capacity [50,55]. However, a larger future study is needed for a more definitive interpretation.

4.4. Strengths and limitations

This study has several strengths. First, we measured trace element concentrations in seminal plasma, which may be a more sensitive and specific biomarker of exposure for studies of sperm quality than trace element concentrations in blood and urine [18]. We also collected seminal plasma at two time points, approximately one week apart, to estimate the variability of trace element concentrations over time and to estimate associations with sperm quality outcomes. Second, we implemented a rigorous laboratory quality control protocol to minimize, identify, and exclude exogenous trace element contamination from seminal plasma specimens in our analysis. Third, we adjusted our models for important covariates to minimize confounding bias. Finally, we used PCA to estimate associations between a mixture of multiple seminal plasma trace elements and sperm quality outcomes.

Despite these strengths, several limitations should be considered when interpreting our findings. The small sample size likely had limited statistical power to detect weak associations and associations with complex mixtures of trace elements using PCA. Therefore, we relaxed statistical significance to $P < 0.10$ to detect hypotheses for future confirmation [38]. Some findings may be due to chance. However, our sample size with 2 observations per participant (n = 19)

was sufficiently powered (~80%) to detect moderate biomarker reliability (ICCs ≥ 0.5) [56]. Still, a larger future study will be needed to confirm the results. We recoded missing cigarette smoking responses as "non-smoker" due to a large proportion of missingness, which may have misclassified some participants and biased the study results. However, we found similar results in a sensitivity analysis when we recoded missing cigarette smoking as "smoker," so any impact was likely modest. We did not collect data about male obesity or alcohol use, which may have confounded some of our results and are important to collect in future studies. Furthermore, we were unable to analyze data for several potentially important seminal plasma trace elements (*i.e.,* Ba, Cr, Co, Pb, U, and V) [33,57–59], due to potential exogenous contamination in our samples, as previously reported [24]. No participants reported occupations with exposure to non-essential trace elements, and background exposure was very low in this study population. This likely contributed to lower ICCs and the results may not be relevant to populations with workplace or other higher levels of exposure than background exposures [60]. Future studies in populations with higher levels of exposure to trace element exposures are necessary to more definitively characterize the reliability of seminal plasma trace element concentrations as biomarkers of exposure. Finally, our study focused on male partners of IVF patients from a single fertility center, which may limit the generalizability of the findings. Although participants were unaware of their seminal plasma trace element concentrations at enrollment, making differential participation, and so our results are internally valid. Men undergoing IVF may differ from the general population in fertility characteristics, health status, and environmental exposures. Therefore, our reliability estimates and effect sizes may not be generalizable to other populations.

4.5. Conclusion

We found that As, Cd, Cu, Mn, Mo, Se, Sr, and Zn concentrations in seminal plasma were moderately reliable biomarkers of exposure. We also found that 2–5 repeated seminal plasma sample collections were needed to reliably measure the average seminal plasma concentration of most trace elements. Our study results suggest, that essential (particularly Se and Cu) and non-essential (particularly As and Cd) seminal plasma trace elements may be important predictors of sperm quality at non-occupational exposure levels in male partners of IVF patients. Our results also suggest that seminal plasma trace element concentrations and their mixtures may be associated with sperm quality. Our study provides novel data on the reliability of seminal plasma trace elements and may guide the design of future studies aimed at informing clinical guidelines to improve reproductive outcomes in male partners of couples undergoing IVF treatment.

Supplementary data

A supplementary file includes unadjusted analyses of associations between seminal plasma trace elements and sperm quality outcomes (Table S1), sensitivity analyses of As and Cd and sperm quality outcomes accounting for smoking (Table S2), and mixture analyses of trace elements and sperm quality outcomes (Table S3). Supplementary figures include a study flowchart (Figure S1), conceptual DAG (Figure S2), principal component scree plot (Figure S3), pairwise correlation matrices (Figure S4), and sensitivity analysis results (Figure S5).

Data availability statement

The data that support the findings of this study are not publicly available due to patient confidentiality concerns but are available from the corresponding author upon reasonable request.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used generative AI tools only to improve language and readability. The authors take full responsibility for the content of the manuscript.

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Authors' contribution

Conceptualization, Victor Y. Fujimoto, Patrick J. Parsons and Michael S. Bloom; methodology, Victor Y. Fujimoto, Aubrey L. Galusha, Patrick J. Parsons and Michael S. Bloom; software, Rooshna Mohsin; validation, Aubrey L. Galusha and Patrick J. Parsons; formal analysis, Rooshna Mohsin and Jenna R. Krall; investigation, Victor Y. Fujimoto, Aubrey L. Galusha, Patrick J. Parsons and Evelyn Mok-Lin; resources, Victor Y. Fujimoto and Patrick J. Parsons; data curation, Celeste D. Butts-Jackson and Michael S. Bloom; writing—original draft preparation, Rooshna Mohsin and Michael S. Bloom; writing—review and editing, Victor Y. Fujimoto, Aubrey L. Galusha, Patrick J. Parsons, Jenna R. Krall, Celeste D. Butts-Jackson and Evelyn Mok-Lin; visualization, Rooshna Mohsin; supervision, Victor Y. Fujimoto, Patrick J. Parsons and Michael S. Bloom; project administration, Victor Y. Fujimoto, Patrick J. Parsons and Michael S. Bloom; funding acquisition, Victor Y. Fujimoto, Patrick J. Parsons and Michael S. Bloom. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

Michael S. Bloom holds the position of Advisory Board Member for *International Journal of Environmental Epidemiology* and has not peer reviewed or made any editorial decisions for this paper.

Ethical statement

The study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the University of California at San Francisco (February 2, 2015/No 14-15440), the University at Albany, State University of New York (March 12, 2015/No 15-F-064-01), and the Wadsworth Center, New York State Department of Health (April 2, 2015/No 15-109).

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