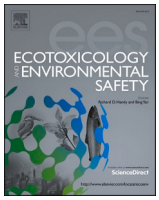
Ecotoxicology and Environmental Safety 251 (2023) 114532

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Sperm mitochondrial DNA copy number mediates the association between seminal plasma selenium concentrations and semen quality among healthy men

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ARTICLE INFO

Edited by Fernando Barbosa

Keywords:
Semen quality
Selenium
MtDNAcn
Mediation analysis

ABSTRACT

Selenium (Se) is essential for successful male reproduction. However, the association of Se status with human semen quality remains controversial and the underlying mechanisms are poorly understood. We measured seminal plasma Se concentrations, sperm mitochondrial DNA copy number (mtDNAcn), and sperm quality parameters among healthy Chinese men screened as potential sperm donors. Linear mixed-effects models were used to investigate the associations of within-subject pooled seminal plasma Se concentrations (n = 1159) with repeated sperm quality parameters (n = 5617); mediation analyses were applied to evaluate the mediating role of sperm mtDNAcn (n = 989). Seminal plasma Se concentrations were positively associated with sperm concentration and total count (both *P* for trend < 0.001). In adjusted models, men in the top vs. bottom quartiles of seminal plasma Se concentrations had 70.1 % (95 % CI: 53.3 %, 88.9 %) and 59.1 % (95 % CI: 40.5 %, 80.2 %) higher sperm concentration and total count, respectively. Meanwhile, we observed inverse associations between seminal plasma Se concentrations and sperm mtDNAcn, and between sperm mtDNAcn and sperm motility, concentration, and total count (all *P* for trend < 0.05). Mediation analyses suggested that sperm mtDNAcn mediated 19.7 % (95 % CI: 15.9 %, 25.3 %) and 23.1 % (95 % CI: 17.4 %, 33.4 %) of the associations between seminal plasma Se concentrations and sperm concentration and total count, respectively. Our findings suggest that Se is essential for male spermatogenesis, potentially by affecting sperm mtDNAcn.

1. Introduction

Selenium (Se) is a critical micronutrient required for many physiological functions in humans, including antioxidant defense system, thyroid hormone metabolism, and immune function (Brown and Arthur, 2001; Rayman, 2012). Low Se status has been linked to increased risks of cognitive decline, poor immune function, and all-cause and cancer mortality (Rayman, 2012). Meanwhile, growing evidence shows that Se

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https://doi.org/10.1016/j.ecoenv.2023.114532
Received 13 August 2022; Received in revised form 6 January 2023; Accepted 7 January 2023
Available online 12 January 2023

is essential for male and female reproductive health (Mistry et al., 2012; Mossa et al., 2018).

Semen quality is commonly used as a proxy to estimate male fertility. Several previous studies have explored the associations between Se concentrations in different biological samples (e.g., urine, blood, and seminal plasma) and semen quality, but the results are still controversial. For example, Zeng et al. (2015) reported that urinary Se concentrations were positively correlated with total sperm count among 394 males. In a similar population consisting of 746 men from an infertility clinic, Wang et al. (2017) found that Se concentrations in seminal plasma were positively associated with sperm concentration. In contrast, Akinloye et al. (2005) revealed an inverse association between serum Se concentrations and total sperm count among 60 idiopathic infertile Nigerian men. Meanwhile, a null association between Se status and human semen quality was also reported (Chai et al., 2022; Shi et al., 2021). These previous studies mostly relied on a single measurement of Se and semen quality, thus measurement error is inevitable, given the abundant high within-individual variation in sperm quality parameters (Chen et al., 2020a; Chiu et al., 2017; Francavilla et al., 2007) and urinary Se concentrations (Chen et al., 2019, 2021). More importantly, less is known about the underlying mechanisms of the relationships between Se status and semen quality, which are critical for improving insights into disease development and prevention.

Mitochondrial DNA copy number (mtDNAcn), a measure of mitochondrial genome abundance (Smith et al., 2021), has been proposed to be a sensitive biomarker for semen quality and mitochondrial dysfunction induced by oxidative stress (Chen et al., 2018; Malik et al., 2013; Rosati et al., 2020). Former studies have shown that sperm mtDNAcn is strongly related to sperm concentration, total count, and sperm motility (May-Panloup et al., 2003; Song and Lewis, 2008; Tian et al., 2014; Zhang et al., 2016). Given that Se has a protective effect against oxidative stress due to its unique antioxidant properties (Kose and Naziroglu, 2014), we suspected that sperm mtDNAcn might play an important mediating role in the association between low Se status and impaired semen quality. Because Se concentrations in seminal plasma are direct markers for Se status in the male reproductive tract (Saaranen et al., 1986), we examined the relationships between seminal plasma Se concentrations, sperm mtDNAcn, and semen quality and explored the mediating role of sperm mtDNAcn among healthy Chinese men screened as potential sperm donors who repeatedly provided semen samples.

2. Materials and methods

2.1. Study design and subjects

From April 2017 to July 2018, healthy men screened as potential sperm donors (n = 1487) were recruited from the Hubei Province Human Sperm Bank, as described elsewhere (Chen et al., 2020a, 2020b; Sun et al., 2019). Briefly, volunteers were eligible if they completed at least a high school degree, aged between 22 and 45 years, and had no sexually transmitted or genetic diseases (Chinese Ministry of Health, 2003). All volunteers completed a questionnaire that gathered information on demographic variables, lifestyle factors, and reproductive history at enrollment (day 0). Physical examinations were also performed to measure waist circumstance, weight, and height. Volunteers provided a semen sample for the initial assessment of semen quality. To avoid selection bias and improve the representativeness of the study population, all volunteers were recruited and asked to provide

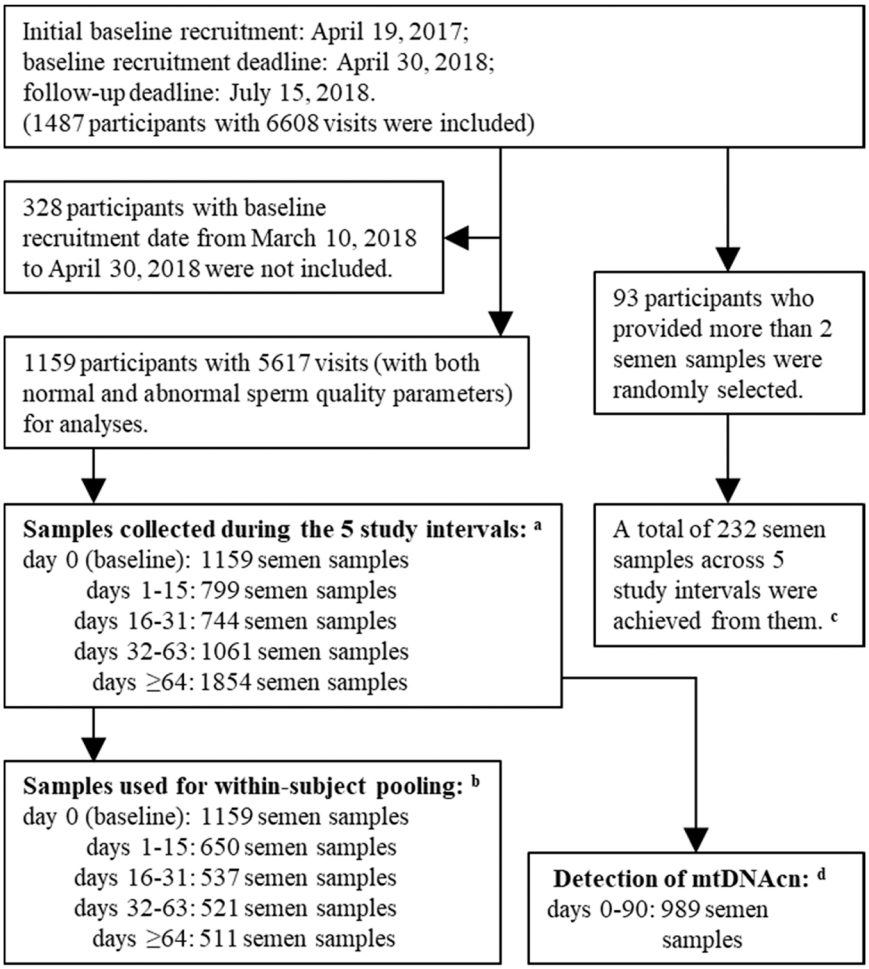

Fig. 1. Study flow. [a] Participants may provide repeated semen samples during follow-up study intervals. [b] The first semen sample collected from each study subject during each pre-defined study interval [i.e., days 0 (baseline), 1–15, 16–31, 32–63, and ≥ 64 from initial recruitment] were pooled in equal volumes. [c] The first semen sample collected from each study subject during each pre-defined study interval [i.e., days 0 (baseline), 1–15, 16–31, 32–63, and ≥ 64 from initial recruitment] were used to investigate the reproducibility of seminal plasma selenium concentrations. [d] Sperm mtDNAcn was measured using the last semen sample collected within 90 days after baseline recruitment.

additional semen samples either for further screening or formal sperm donation during the following 4 study intervals (e.g., 1–15, 16–31, 32–63, and ≥ 64 days since initial recruitment) (Chen et al., 2021). Finally, 1159 volunteers with 5617 semen examinations were included in our subsequent analyses (Fig. 1).

2.2. Semen collection

Semen specimens were gathered at baseline and during each follow-up visit into a trace-element-free sterile polypropylene container by masturbation in a private room at the Hubei Province Human Sperm Bank. We measured sperm quality parameters for each semen sample. Due to the preciousness of semen samples and the considerable within-person variability in seminal plasma Se concentrations, we pooled the first semen specimen collected from each participant within pre-defined study intervals [i.e., days 0 (baseline), 1–15, 16–31, 32–63, and ≥ 64 since initial recruitment] in equal volumes to reflect the average Se status over an approximately 3-month period (Perrier et al., 2016), which corresponds to the duration of spermatogenesis (Fig. 1).

2.3. Measurement of sperm quality parameters

Semen volume (mL), sperm concentration (million/mL), and motility (%) were assessed by qualified laboratory technicians according to the World Health Organization (WHO) guidelines (WHO, 1999), as reported before (Chen et al., 2020a, 2020b; Sun et al., 2019). A weighing method was adopted to measure semen volume. Sperm concentration and motility were assessed using an optical microscope and a cytometer. Total sperm count (million per ejaculation) was calculated as semen volume × sperm concentration (WHO, 2010). Daily internal quality controls were accomplished to guarantee that interday and intraday variations were less than 10 %.

2.4. DNA extraction and mtDNAcn measurement

To prospectively investigate the association between seminal plasma Se concentrations and sperm mtDNAcn, DNA samples were extracted from the last semen specimen taken within 90 days since baseline recruitment utilizing the TIANamp Genomic DNA Kit (TIANGEN Biochemical Technology Co., Ltd, Beijing, China). The extracted DNA samples were quantified utilizing the Nanodrop spectrophotometer (ND-1000; Thermo Scientific Inc. DE, USA) and then immediately stored at − 80 ℃ refrigerators until mtDNAcn measurement. Sperm mtDNAcn in the DNA samples was determined by real-time fluorescent quantitative PCR (RT-qPCR). Detailed information is presented in Supplementary Material and can also be found in our previous studies (Hou et al., 2019; Liu et al., 2022; Sun et al., 2022). Finally, a total of 1228 DNA samples were successfully extracted and determined for sperm mtDNAcn.

2.5. Determination of seminal plasma Se concentrations

Seminal plasma Se concentrations were quantified utilizing inductively coupled plasma mass spectrometry (Agilent 7700x ICP-MS; Agilent Technologies, USA), using the method described previously (Wan et al., 2019; Wang et al., 2017). Concisely, a 150-μL within-subject pooled seminal plasma was transferred to a 5-mL trace element-free polyethylene tube and then acidized by 2.85 mL 1.0 % HNO_3. Spiked within-subject pooled seminal plasma and standard reference materials (SRMs) 2670a and 1640a were employed as reference controls. The limit of quantification (LOQ) for Se in seminal plasma was 1.10 μg/L. All specimens had values higher than the lowest detectable level, and the measured concentrations were substituted with the mean concentration + 3 × standard deviation (SD) for those with measured concentrations higher than this value (Long et al., 2019).

2.6. Within-person variability of seminal plasma Se concentrations

To assess the reproducibility of seminal plasma Se concentrations, we measured Se concentrations in 232 repeated seminal plasma specimens collected from 93 randomly selected volunteers across the pre-defined 5 study intervals [i.e., days 0 (baseline), 1–15, 16–31, 32–63, and ≥ 64 since initial recruitment] (Fig. 1).

2.7. Statistical analyses

Descriptive analyses were performed for volunteers' demographics and the distribution of sperm mtDNAcn, seminal plasma Se concentrations, and sperm quality parameters. After the assessment of normality using the Shapiro-Wilk test, sperm mtDNAcn, seminal plasma Se concentrations, and sperm quality parameters were skewed and thus $\log_{10}$-transformed to satisfy the normality assumptions in subsequent analyses. The reproducibility of seminal plasma Se concentrations was assessed based on the intraclass correlation coefficients (ICCs, 0–1), which were calculated as the between-individual variance divided by the total variance (Rosner, 1999).

To evaluate potential dose-response relationships, we categorized study volunteers into quartiles (Q1: < 23.73 μg/L; Q2: 23.73- < 29.79 μg/L; Q3: 29.79- < 36.61 μg/L; Q4: ≥ 36.61 μg/L) according to the distribution of within-subject pooled seminal plasma Se concentrations. Linear mixed-effects models were used to analyze the associations between the quartiles of within-subject pooled seminal plasma Se concentrations and repeated sperm quality parameters (Verbeke, 2000). Linear regression models were constructed to investigate the associations between the quartiles of within-subject pooled seminal plasma Se concentrations and sperm mtDNAcn, and between the quartiles of sperm mtDNAcn and within-subject average sperm quality parameters. Linear trends were evaluated by modeling the quartiles of seminal plasma Se concentrations or sperm mtDNAcn as continuous variables (i.e., 1–4). Log-transformed seminal plasma Se concentrations and sperm mtDNAcn were also analyzed as continuous variables. The formula [$(10^{\beta} - 1) \times 100$] was used to convert regression coefficients (95 % CI) into percentage change (95 % CI). Confounders were selected a *priori* relied on our prior studies (Chen et al., 2021; Wang et al., 2017), including demographic variables (i.e., age and education level), lifestyle factors (i.e., abstinence time, smoking status, and alcohol consumption), reproductive history (i.e., ever fathered a child), physical examination (i.e., BMI and waist-hip ratio), liquefaction time, and sampling seasons (i.e., only adjusted in the linear mixed model). To explore potential non-linear dose-response relationships of seminal plasma Se concentrations with sperm mtDNAcn and sperm quality parameters, restricted cubic splines were constructed by modeling log-transformed Se concentrations as continuous variables (Desquilbet and Mariotti, 2010).

Mediation analyses were implemented to explore whether sperm mtDNAcn mediated the association between seminal plasma Se concentrations and sperm quality parameters (*medeff* command in Stata) (Hicks and Tingley, 2012). We utilized linear regression models to examine exposure-outcome (i.e., within-individual pooled Se concentrations and within-subject average semen quality; total effects), exposure-mediator (i.e., within-individual pooled Se concentrations and sperm mtDNAcn), and exposure-mediator-outcome associations (i.e., within-individual pooled Se concentrations, sperm mtDNAcn, and within-subject average semen quality; indirect effects). The mediated proportion by sperm mtDNAcn was defined as the ratio of the indirect effect to the total effect.

Several sensitivity analyses were performed to assess the robustness of associations between seminal plasma Se concentrations and semen quality. First, we additionally adjusted for seminal plasma concentrations of cobalt, copper, iron, manganese, molybdenum, and zinc to account for the joint associations of redox metals (Zoroddu et al., 2019). Second, we used within-individual average sperm quality parameters across all visits to evaluate the influence of different sampling numbers

between participants. Third, we excluded semen samples that were not used for within-subject pooling to avoid potential bias. Fourth, we assessed the potential influence of changes in lifestyle and diet factors by excluding participants who did not reach donation criteria at recruitment. Fifth, we used sperm quality parameters measured in the single semen sample that was tested for mtDNAcn. Finally, we used untransformed Se concentrations in restricted cubic spline models. All analyses were conducted utilizing Stata 15.0 (Stata Corp, College Station, TX, USA) and R 4.0.2 (R Development Core Team).

3. Results

3.1. Characteristics of volunteers

The 1159 volunteers had a mean (SD) age, abstinence duration, waist-hip ratio, and BMI of 28.1 (5.3) years, 6.2 (3.3) days, 0.8 (0.1), and 22.8 (3.3) kg/m^2, respectively The majority of volunteers had less than a college degree (65.3 %), never fathered a child (72.9 %), never smoked (54.0 %), and drank alcohol occasionally (60.2 %) (Table 1).

3.2. Distribution of Se concentrations, sperm mtDNAcn, and semen quality

Se concentrations were detected in all pooled within-subject samples (n = 1159). The median (interquartile range, IQR) Se concentrations in seminal plasma were 29.8 (23.7–36.6) μg/L. Among 1159 volunteers recruited in the current study, 989 were measured for mtDNAcn. The median (IQR) sperm mtDNAcn was 0.8 (0.5–1.2). We collected 5617 semen samples from 1159 men and the proportion of semen measurements that were lower than the WHO (2010) reference values ranged from 4.0 % to 7.0 %. The median (IQR) semen volume, sperm concentration, total count, total motility, and progressive motility were 2.8 (2.0–4.0) mL, 61.0 (42.0–68.0) million/mL, 158.4 (113.1–224.0) million per ejaculate, 64.0 (54.0–67.0) %, and 60.0 (50.0–65.0) %, respectively (Table 2).

3.3. Reproducibility of seminal plasma Se concentrations

The between-subject variance (0.016; 64 %) was higher than the within-subject variance (0.009; 36 %) for repeated seminal plasma Se concentrations among 93 men with 232 measurements. The estimated ICC for repeated Se measurements was 0.64 (Table S3).

3.4. Associations between Se concentrations, sperm mtDNAcn, and sperm quality parameters

In crude models, we found positive dose-response relationships between within-individual pooled seminal plasma Se concentrations and sperm concentration and total count (both P for trend < 0.001). In adjusted models, participants in the top vs. bottom quartiles of within-individual pooled seminal plasma Se concentrations had 70.1 % (95 % CI: 53.3 %, 88.9 %) and 59.1 % (95 % CI: 40.5 %, 80.2 %) higher sperm concentration and total count, respectively; these inverse associations persisted when we modeled seminal plasma Se concentrations as continuous variable (All $P < 0.05$) (Table 3). These associations were materially unchanged when we further adjusted for other redox metals (Table S4), when we used within-individual average sperm quality parameters or sperm quality parameters measured in the single semen sample that was tested for mtDNAcn (Table S5-S6), and when we excluded semen samples that were not used for within-subject pooling process or men who did not meet donation criteria at baseline recruitment (Table S7-S8). These positive dose-response relationships were further demonstrated in cubic spline analyses when seminal plasma Se concentrations were modeled as continuous variables with or without logarithmic transformation (Fig. 2 and Fig. S1).

We found inverse dose-response relationships between within-individual pooled Se concentrations and sperm mtDNAcn both in crude and adjusted linear regression models (both P for trend < 0.05),

Table 1
Baseline characteristics of the participants [n (%) or mean ± SD]. [a].

Characteristics	Participants included in the current analysis (n = 1159)	Stratified by seminal plasma Se concentration			
		Q1 (n = 290)	Q2 (n = 290)	Q3 (n = 290)	Q4 (n = 289)
Age, years	28.1 ± 5.3	27.1 ± 4.5	27.6 ± 5.3	27.9 ± 5.1	29.6 ± 5.9
Education level					
Less than college	757 (65.3)	211 (72.8)	187 (64.5)	184 (63.4)	175 (60.6)
College and above	402 (34.7)	79 (27.2)	103 (35.5)	106 (36.6)	114 (39.4)
Ever fathered a child					
No	845 (72.9)	233 (80.3)	217 (74.8)	210 (72.4)	185 (64.0)
Yes	314 (27.1)	57 (19.7)	73 (25.2)	80 (27.6)	104 (36.0)
Smoking status					
Never	626 (54.0)	125 (43.1)	166 (57.2)	163 (56.2)	172 (59.5)
Former	84 (7.3)	21 (7.2)	24 (8.3)	17 (5.9)	22 (7.6)
Current	449 (38.7)	144 (49.7)	100 (34.5)	110 (37.9)	95 (32.9)
Alcohol consumption					
Never	287 (24.8)	69 (23.8)	73 (25.2)	68 (23.4)	77 (26.6)
Occasional	698 (60.2)	174 (60.0)	176 (60.7)	175 (60.3)	173 (59.9)
Former	12 (1.0)	4 (1.4)	0 (0.0)	6 (2.1)	2 (0.7)
Current	162 (14.0)	43 (14.8)	41 (14.1)	41 (14.1)	37 (12.8)
BMI, kg/m^2	22.8 ± 3.3	22.5 ± 3.4	22.8 ± 3.3	22.8 ± 3.3	23.3 ± 3.1
Waist-hip ratio	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
Abstinence time, days[b]	6.2 ± 3.3	6.4 ± 3.9	5.9 ± 2.3	6.3 ± 3.2	6.3 ± 3.8
Liquefaction time, minutes[b]	24.8 ± 10.6	23.6 ± 10.1	24.9 ± 10.7	24.8 ± 10.6	25.5 ± 10.8
Season at semen examination[b]					
Spring (Mar-May	1086 (19.3)	190 (19.3)	275 (19.4)	297 (19.7)	324 (19.0)
Summer (Jun-Aug)	2049 (36.5)	330 (33.4)	512 (36.2)	560 (37.1)	647 (37.9)
Autumn (Sept-Nov)	1665 (29.6)	293 (29.7)	413 (29.2)	426 (28.2)	533 (31.2)
Winter (Dec-Feb)	817 (14.6)	174 (17.6)	214 (15.1)	225 (14.9)	204 (11.9)

Abbreviations: BMI, body mass index; Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile; Se, selenium.

[a] One participant had missing data on BMI, 1 on waist circumference, and 4 on the history of having ever fathered a child; we used the median imputation method to handle missing information. Demographic characteristics across quartiles of seminal plasma Se concentration were compared utilizing Kruskal-Wallis or χ^2 tests where appropriate.

[b] The numbers were caculated for semen specimens provided by all participants throughout the study period.

Table 2
Distribution of within-individual pooled seminal plasma Se concentrations (μg/L), sperm mtDNAcn, and sperm quality parameters.

Parameters	n (%)[a]	Geometric mean	Mean	P10	Median	P25-P75	P90
Se (n = 1159)	-	29.2	30.9	18.8	29.8	23.7–36.6	44.4
MtDNAcn (n = 989)	-	0.8	1.0	0.3	0.8	0.5–1.2	1.9
Sperm quality parameters (n = 5617)							
Semen volume	309 (5.5)	2.8	3.1	2.0	2.8	2.0–4.0	4.8
Sperm concentration	241 (4.3)	49.5	56.6	23.0	61.0	42.0–68.0	82.0
Total sperm count	395 (7.0)	137.1	171.2	52.8	158.4	113.1–224.0	294.0
Total motility	345 (6.1)	58.4	60.3	43.0	64.0	54.0–67.0	72.0
Progressive motility	222 (4.0)	55.1	57.2	40.0	60.0	50.0–65.0	69.0

Abbreviations: LOQ, limits of quantification; mtDNAcn, mitochondrial DNA copy number; Se, selenium.
[a] n (%) refers to the proportion of semen samples below WHO (2010) reference values using all semen measurements.

Table 3
Percentage change [%Δ (95 % CI)] or regression coefficients (95 % CI) of within-subject pooled seminal plasma Se concentration in relation to repeated sperm quality parameters based on linear mixed-effects models (n = 5617) and sperm mtDNAcn based on linear regression models (n = 989).

Sperm parameters	Quartiles of seminal plasma Se concentration[a]					Per one-unit increment in natural log-transformed Se (μg/L)[b]
	Q1	Q2	Q3	Q4	*P* for trend	
Semen volume						
Crude model	Ref	4.8 (−2.0, 12.0)	3.1 (−3.6, 10.2)	-3.6 (−10.0, 3.3)	0.393	0.02 (−0.06, 0.09)
Adjusted model[c]	Ref	4.0 (−2.6, 11.1)	1.0 (−5.4, 7.8)	-6.5 (−12.7, 0.2)	0.079	-0.02 (−0.09, 0.05)
Sperm concentration						
Crude model	Ref	38.0 (25.1, 52.3)	50.4 (36.4, 65.9)	78.7 (61.4, 97.9)	**<0.001**	0.70 (0.60, 0.81)*
Adjusted model[c]	Ref	36.5 (23.7, 50.7)	45.9 (32.3, 61.0)	70.1 (53.3, 88.9)	**<0.001**	0.65 (0.54, 0.76)*
Total sperm count						
Crude model	Ref	44.0 (27.7, 62.3)	54.3 (36.9, 73.8)	71.6 (51.6, 94.2)	**<0.001**	0.72 (0.59, 0.85)*
Adjusted model[c]	Ref	41.5 (25.8, 59.2)	46.9 (30.6, 65.3)	59.1 (40.5, 80.2)	**<0.001**	0.63 (0.50, 0.76)*
Total motility						
Crude model	Ref	-0.8 (−5.1, 3.7)	-1.5 (−5.7, 2.9)	-2.7 (−6.9, 1.8)	0.229	-0.05 (−0.10, −0.01)*
Adjusted model[c]	Ref	-1.1 (−5.2, 3.3)	-1.0 (−5.1, 3.4)	-1.7 (−6.0, 2.8)	0.475	-0.04 (−0.09, 0.01)
Progressive motility						
Crude model	Ref	-0.9 (−5.4, 3.9)	-1.2 (−5.6, 3.5)	-2.1 (−6.7, 2.7)	0.386	-0.05 (−0.10, 0.004)
Adjusted model[c]	Ref	-1.2 (−5.6, 3.4)	-0.6 (−5.0, 4.0)	-1.1 (−5.7, 3.7)	0.690	-0.04 (−0.09, 0.02)
Sperm mtDNAcn						
Crude model	Ref	-2.9 (−14.4, 10.2)	-6.4 (−17.5, 6.2)	-17.5 (−27.3, −6.4)	**0.003**	-0.19 (−0.32, −0.05)*
Adjusted model[d]	Ref	-0.4 (−12.2, 13.0)	-3.3 (−14.8, 9.7)	-13.0 (−23.6, −1.1)	**0.032**	-0.13 (−0.27, 0.01)

Abbreviations: CI, confidence interval; mtDNAcn, mitochondrial DNA copy number; Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile; Se, selenium.
* $P < 0.05$.
[a] The values were percentage change [%Δ (95 % CI)].
[b] The values were regression coefficients (95 % CI).
[c] Models were adjusted for age, body mass index, waist-hip ratio, education levels (less than undergraduate or undergraduate and above), ever fathered a child (yes or no), smoking status (never, former, or current), alcohol consumption (never, former, occasional, or current), abstinence time, sampling seasons (spring, summer, fall, or winter), and liquefaction time.
[d] Models were adjusted for age, body mass index, waist-hip ratio, education levels (less than undergraduate or undergraduate and above), ever fathered a child (yes or no), smoking status (never, former, or current), alcohol consumption (never, former, occasional, or current), average abstinence time, and average liquefaction time.

which, again, were further confirmed in cubic spline analyses when we modeled seminal plasma Se concentrations as continuous variables (Fig. 2 and Fig. S1). In adjusted models, participants in the top vs. bottom quartiles of within-subject pooled Se concentrations had a 13.0 % (95 % CI: −23.6 %, −1.1 %) lower sperm mtDNAcn (Table 3). Comparing the top vs. bottom sperm mtDNAcn quartiles, participants exhibited lower sperm concentration [− 40.8 % (95 % CI: −46.4 %, −34.8 %); *P* for trend < 0.001], total count [− 41.2 % (95 % CI: −47.8 %, −33.8 %); *P* for trend < 0.001], total motility [− 8.1 % (95 % CI: −12.6 %, −3.4 %); *P* for trend = 0.010]), and progressive motility [− 8.6 % (95 % CI: −13.4 %, −3.7 %); *P* for trend = 0.008] (Table S9). These results remained consistent when we used sperm quality parameters measured in the single semen sample that was tested for mtDNAcn (Table S10).

Mediation analyses showed that sperm mtDNAcn mediated 19.7 % (95 % CI: 15.9 %, 25.3 %) of the association between Se concentrations and sperm concentration and 23.1 % (95 % CI: 17.4 %, 33.4 %) between Se concentrations and total sperm count (Fig. 3). Similar results were observed when we used sperm quality parameters measured in the single semen sample that was tested for mtDNAcn (Fig. S2).

4. Discussion

Among 1159 healthy men who had 5617 measurements of sperm quality parameters, we found positive dose-response relationships between seminal plasma Se concentrations and sperm concentration and total count. Besides, we observed inverse relationships between seminal plasma Se concentrations and sperm mtDNAcn, and between sperm mtDNAcn and sperm motility, concentration, and total count. Mediation analyses demonstrated that nearly 20 % of the associations between Se concentrations and sperm concentration and total count were mediated by sperm mtDNAcn.

Se is critical for testosterone biosynthesis and the normal development of spermatozoa (Mistry et al., 2012). Consistent with this view, our results indicated that seminal plasma Se concentrations were positively related to sperm concentration and total sperm count in a dose-dependent manner. In support of our findings, Liu et al. (2020) revealed positive relationships between seminal plasma Se

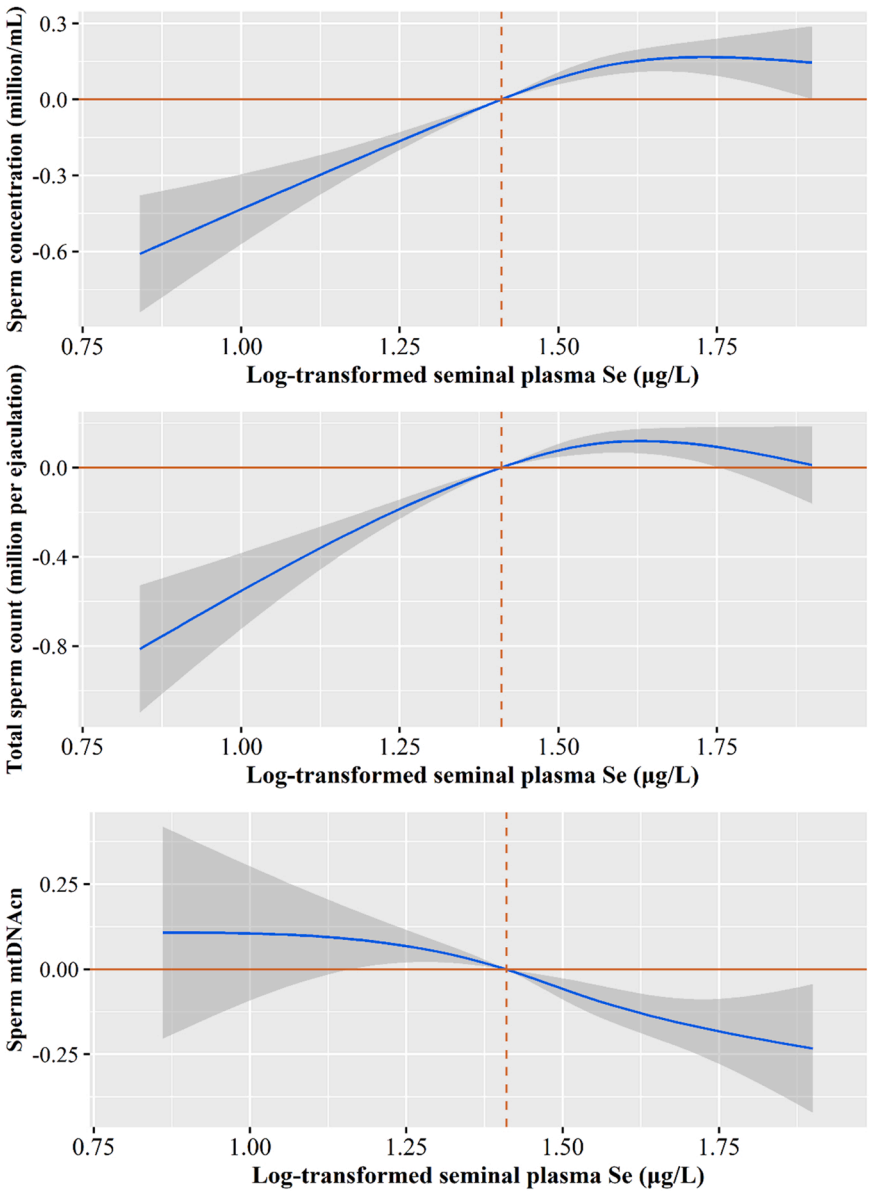

Fig. 2. Restricted cubic splines for the associations of within-subject pooled seminal Se concentrations (log_{10}-transformed) with repeated sperm quality parameters (n = 1159 subjects, 5617 semen samples) and sperm mtDNAcn (n = 989 subjects, 989 semen samples). The reference values were set at the 25th percentile (i.e., 1.41 μg/L for Se). The blue solid lines are the effect estimates, and the shadowed parts are the 95 % confidence intervals. In the analyses between seminal plasma Se and sperm quality parameters, models were adjusted for age, body mass index, waist-hip ratio, education levels (less than undergraduate or undergraduate and above), ever fathered a child (yes or no), smoking status (never, former, or current), alcohol consumption (never, former, occasional, or current), abstinence time, sampling seasons (spring, summer, fall, or winter), liquefaction time, and seminal plasma redox metals (i.e., cobalt, copper, iron, manganese, molybdenum, and zinc). In the analysis of the association between seminal plasma Se and sperm mtDNAcn, the model was adjusted for age, body mass index, waist-hip ratio, education levels (less than undergraduate, or undergraduate and above), ever fathered a child (yes or no), smoking status (never, former, or current), alcohol consumption (never, former, occasional, or current), average abstinence time, average liquefaction time, and seminal plasma redox metals (i.e., cobalt, copper, iron, manganese, molybdenum, and zinc). Abbreviations: Se, selenium.

concentrations and sperm concentration and total count among 1136 men seeking a fertility consultation in hospitals. Zeng et al. (2015) showed a positive relationship between urinary Se concentrations and total sperm count among 394 males from an infertility clinic. Calogero et al. (2021) found higher Se concentrations in blood and seminal plasma among 48 normozoospermic men than 131 men with asthenozoospermia or oligozoospermia. Eroglu et al. (2014) reported that seminal plasma and serum Se concentrations were positively associated with sperm concentration, motility, and morphology among 59 men attending an infertility clinic. In a double-blind randomized study, Safarinejad and Safarinejad (2009) reported that Se supplementation improved semen quality among 68 infertile men. However, controversial findings were also reported among healthy men and male partners of couples from infertility clinics (Akinloye et al., 2005; Hawkes and Turek, 2001). The inconsistency between studies is not unexpected given the differences in Se status, biological matrices (e.g., urine, blood, and seminal plasma), the composition of the study populations (e.g., infertile vs. healthy men), and sample size. More importantly, former studies mostly relied on a single determination of Se and semen quality, which may have led to measurement error. Among a subgroup of 93 volunteers who provided 232 semen samples, we evaluated, for the first time, the variability of Se concentrations in seminal plasma. We found that seminal plasma Se concentrations exhibited fair to good reproducibility (Rosner, 1999), probably due to the changes in dietary intake and physiological metabolism (Hawkes et al., 2008). Our novel findings indicate potential exposure misclassification in previous studies measuring Se in a single semen sample. Substantial studies also reported considerable within-individual variability in sperm quality parameters (Chen et al., 2020a, 2020b; Chiu et al., 2017) and urinary Se concentrations (Chen et al., 2019, 2021). All these findings emphasize the importance of collecting repeated samples.

Se is an important ingredient of glutathione peroxidase enzymes (Mirnamniha et al., 2019) and is considered to shield germline cells from oxidative damage (Ursini et al., 1999), which, thus, plays a protective role against oxidative stress. Sperm mtDNAcn has been proposed to be a sensitive biomarker for reduced semen quality and mitochondrial dysfunction induced by oxidative stress (Passos et al., 2007). In the present study, we found inverse relationships between seminal plasma Se concentrations and sperm mtDNAcn, and between sperm mtDNAcn and sperm motility, concentration, and total count. Mediation analyses further revealed that sperm mtDNAcn mediated nearly 20 % of the association between seminal plasma Se concentrations and sperm

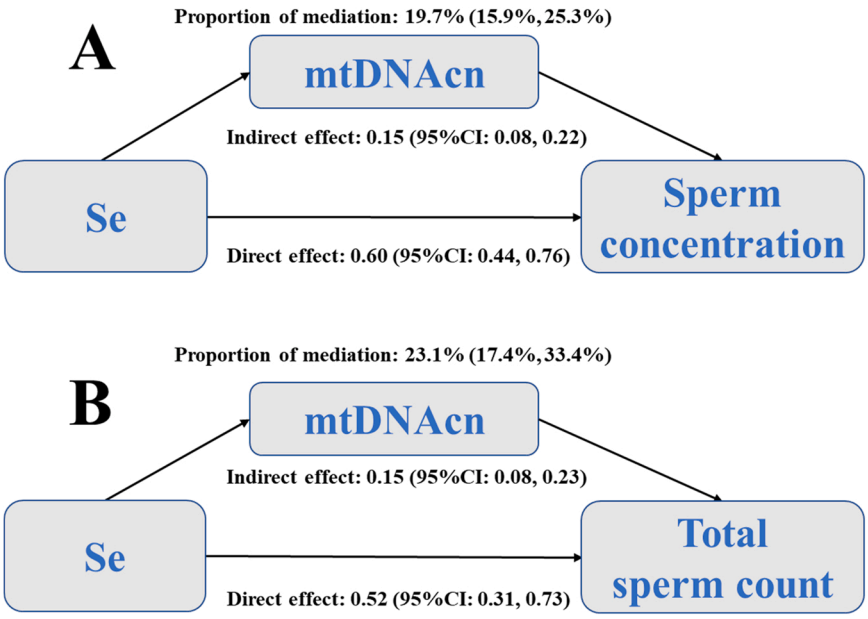

Fig. 3. The estimated proportions of associations between within-individual average seminal plasma Se concentrations and sperm quality parameters mediated by sperm mtDNAcn (n = 989). Models were adjusted for age, body mass index, waist-hip ratio, education levels (less than undergraduate or undergraduate and above), ever fathered a child (yes or no), smoking status (never, former, or current), alcohol consumption (never, former, occasional, or current), average abstinence time, average liquefaction time, and seminal plasma redox metals (i.e., cobalt, copper, iron, manganese, molybdenum, and zinc). Abbreviations: CI, confidence interval; mtDNAcn, mitochondrial DNA copy number; Se, selenium.

concentration and total count. We suspect that higher Se status could suppress oxidative stress, as evidenced by the activity of the antioxidant selenoenzyme glutathione peroxidase (Hawkes and Alkan, 2010; Liu et al., 2014; Zhang et al., 2020), which may lead to fewer sperm mtDNAcn and eventually reduced the harmful effect induced by oxidative stress on spermatogenesis (Bisht et al., 2017).

This study has some strengths. First, we collected repeated semen samples from each volunteer to measure seminal plasma Se concentrations and sperm quality parameters, which should have reduced measurement error (Auger et al., 2000; Chen et al., 2020a, 2020b). Second, we determined Se concentrations in seminal plasma that could directly reflect Se status in the male reproductive tract. Third, we recruited healthy men screened as potential sperm donors who would be more representative than participants recruited from infertility clinics. Nonetheless, there are also several limitations. First, we measured sperm mtDNAcn at a single time point, which might have led to measurement error. Second, we measured seminal plasma Se concentrations in within-subject pooled samples to reflect the average Se status across the duration of spermatogenesis, which, however, made it impossible to determine the potential window of susceptibility (Chen et al., 2021). Third, our analyses were restricted to men who were measured for mtDNAcn, which may have led to selection bias since we observed higher total sperm count and progressive motility among participants who were measured for mtDNAcn than those who weren't. Fourth, although we have adjusted for many covariates, residual confounding from other unmeasured covariates or environmental pollutants (e.g., diet and toxic metals) cannot be fully excluded. Finally, as with any observational study, our study cannot demonstrate causality.

5. Conclusions

Relied on repeated measurements study design, we observed positive dose-response relationships between seminal plasma Se concentrations and sperm concentration and total count, which was partly mediated by sperm mtDNAcn. Our findings highlight the importance of Se in improving semen quality and provide an original clue of the underlying mechanism related to sperm mtDNAcn.

CRediT authorship contribution statement

All authors fulfill the criteria for authorship. **Heng-Gui Chen** analyzed the data. **Heng-Gui Chen** and **Yi-Xin Wang** drafted the original manuscript. **Weimin Ye** and **Yi-Xin Wang** lead the study design, study conception, analysis plan, and interpretation of findings. **Bin Sun, Ying-Jun Chen, Cheng-Liang Xiong, Tian-Qing Meng**, and **Peng Duan** contributed to the acquisition of data. **Bin Sun** and **Fuxin Lin** validated the accuracy of data analysis with a technical review. **An Pan, Zhijian Hu,** and **Carmen Messerlian** reviewed and edited the manuscript. All authors appraised the manuscript and approved the publication.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by grants from the Natural Science Foundation of Fujian Province (No. 2022J05136), the National Natural Science Foundation of China [No. 81903281], the National Postdoctoral Program for Innovative Talents (No. BX201700087), and the funding from the First Affiliated Hospital of Fujian Medical University (No. YJRC3883). We also sincerely thank all the participants for participating in this study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.ecoenv.2023.114532.

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