Environmental Research 263 (2024) 120086

Contents lists available at ScienceDirect

Environmental Research

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

Exploring the association between urinary bisphenol A, S, and F levels and semen quality parameters: Findings from Led-Fertyl cross-sectional study

María Ángeles Martínez [a,b,c,d,**], Albert Salas-Huetos [a,b,c,e,f], María Fernández de la Puente [a,b,c], Cristina Valle-Hita [a,b,c], Montse Marquès [b,g], Claudia Del Egido-González [a,b], Estefanía Davila-Cordova [a,b,c], Cristina Mestres [a,b,h], Maria Skaalum Petersen [i,j], Nancy Babio [a,b,c,*,1], Jordi Salas-Salvadó [a,b,c,1]

[a] *Institut d'Investigació Sanitària Pere Virgili (IISPV), Reus, Spain*
[b] *Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Alimentació, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, Reus, Spain*
[c] *Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Institute of Health Carlos III, Madrid, Spain*
[d] *Department of Pharmacology, therapeutics and Toxicology, Faculty of veterinary. Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193, Catalonia, Spain*
[e] *Universitat Rovira i Virgili, Departament de Ciències Mediques Bàsiques, Unitat de Medicina Preventiva, Alimentació, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, Reus, Spain*
[f] *Department of Nutrition, Harvard T.H. Chan School of Public Health, Harvard University, Boston, MA, USA*
[g] *Universitat Rovira i Virgili, Departament de Ciències Mèdiques Bàsiques, Laboratori de Toxicologia i Salut Ambiental. Center of Environmental, Food and Toxicological Technology—TecnATox. Reus, Spain*
[h] *Hospital Universitari Sant Joan de Reus, Universitat Rovira i Virgili, IISPV, Alimentaciò, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, Reus, Spain*
[i] *University of the Faroe Islands, Tórshavn, Faroe Islands*
[j] *Department of Research, the National Hospital of the Faroe Islands, Tórshavn, Faroe Islands*

ARTICLE INFO

Keywords:
Endocrine disruptors
Semen quality
Infertility
Bisphenols
Chemical analogues

ABSTRACT

Infertility is recognized as a multifaceted condition affecting approximately 15% of couples globally, influenced by various factors including genetic predisposition and environmental exposures. Among these environmental factors, bisphenol A (BPA) emerges as a prominent Endocrine-disrupting chemical (EDCs) widely distributed, leading to chronic human exposure in daily life. As regulations on BPA became more stringent, alternative substances such as bisphenol S (BPS) and bisphenol F (BPF) have emerged. Animal studies have demonstrated a dose-dependent decline in fertility and embryotoxicity following chronic exposure to BPA. However, literature data on human studies are limited and heterogeneous. Additionally, even less is known about the relationship between exposure to the BPA analogues (BPS and BPF) and sperm quality. Therefore, the present study aimed to examine the association between urinary concentrations of BPA, BPF, and BPS and semen quality parameters among 195 adult Spanish men from the Led-Fertyl study cohort using multiple linear regression models adjusted by potential confounding variables. Our results revealed an inverse association between log-transformed creatinine-adjusted concentration (ng/mg) of BPA and BPF levels and the percentage of sperm vitality (β: 3.56 %; 95%CI: 6.48 to −0.63 and β: 4.14 %; 95%CI: 6.97 to −1.31; respectively). Furthermore, participants in the highest quartile of BPA and BPF urinary concentration exhibited lower sperm vitality compared to those in the lowest quartile (β: 6.90 %; 95%CI: 11.60 to −2.15 and β: 9.68 %; 95%CI: 14.43 to −4.94; respectively). These results supply epidemiological evidence establishing a relationship between bisphenols urine exposure and sperm quality, suggesting that a re-evaluation of the overall safety of BPA alternatives is warranted.

---

[*] Corresponding author. Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Alimentació, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, Reus, Spain. C/Sant Llorenç, 21. 43201. Reus, Spain.

[**] Corresponding author. Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Alimentaciò, Nutrició, Desenvolupament i Salut Mental ANUT-DSM, Reus, Spain. C/Sant Llorenç, 21. 43201. Reus, Spain.

E-mail addresses: mariaangeles.martinez@iispv.cat (M.Á. Martínez), nancy.babio@urv.cat (N. Babio).

[1] Co-senior.

https://doi.org/10.1016/j.envres.2024.120086
Received 7 June 2024; Received in revised form 13 September 2024; Accepted 28 September 2024

1. Introduction

Infertility rates have notably increased in recent years, affecting approximately 15% of the global population, with male factors contributing to nearly half of these cases (Agarwal et al., 2021). Infertility is widely recognized as a multifactorial condition, influenced by various factors including genetic predisposition and environmental exposures (Diamanti-Kandarakis et al., 2009; Salas-Huetos et al., 2017). These environmental exposures encompass a spectrum of elements encountered in daily life, collectively known as chemical exposome, with the prevalence of exposure to Endocrine-disrupting chemicals (EDCs) being notable (Diamanti-Kandarakis et al., 2009; Thomas Zoeller et al., 2012). The presence of EDCs in our environment has raised concerns due to their potential impact on reproductive health, with studies linking them to a decline in human sperm concentration over the past 40 years (Agarwal et al., 2021; Levine et al., 2023; Vander Borght and Wyns, 2018).

Among more than 800 recognized EDCs, bisphenol A (BPA) stands out as a well-known synthetic compound extensively distributed and mainly present in polycarbonate plastic products and epoxy resins, which have been in use since the mid-20th century (Gimeno et al., 2015; Matuszczak et al., 2019; Ni et al., 2024). The widespread presence of BPA has raised significant concerns regarding its potential impact on human health. Exposure to BPA in humans, primarily occurs through food ingestion, although dermal contact and inhalation routes are also plausible (Gimeno et al., 2015; Martínez et al., 2018; Ribeiro et al., 2017). As an endocrine disruptor, BPA has the capability to disrupt hormonal homeostasis and detrimentally affect developmental, immunological, neurological, and reproductive functions, especially during the vulnerable foetal development stage (Ma et al., 2019; Martínez et al., 2021; Ni et al., 2022; Ribeiro et al., 2017; Sabuz Vidal et al., 2021).

BPA is primarily excreted through urine, with an elimination rate typically occurring within hours (Völkel et al., 2002). Biomonitoring studies have consistently demonstrated the widespread prevalence of BPA exposure among the general population (Calafat et al., 2008; Koch et al., 2012; Martínez et al., 2021).

Regulatory agencies worldwide have implemented stringent measures to mitigate BPA exposure, such as prohibiting certain products, particularly those involving plastic baby items, and establishing migration limits in food packaging materials. The gradual reduction of the tolerable daily intake (TDI) of BPA from 50 μg/kg body weight/day in 2006, 4 μg/kg body weight/day in 2015 to 0.2 ng/kg body weight/day in 2023 highlights the high importance of comprehending its health impacts on the human population (EFSA, 2004, 2015, 2023). In response to restrictions on BPA, alternative substances such as bisphenol S (BPS) and bisphenol F (BPF) have emerged. It appears that these emerging analogues may exhibit endocrine-disrupting properties similar to or even greater than those of BPA itself *in vitro,* however, they are currently being widely used (Martínez et al., 2020; Ullah et al., 2019b).

Animal studies have demonstrated a dose-dependent decline in fertility and embryo toxicity following chronic exposure to BPA. Male rodents exposed to oral BPA exhibit impaired reproductive function, characterized by reduced testosterone levels (Jin et al., 2013), compromised Sertoli cell function (Jin et al., 2013; Li et al., 2011), and disrupted sperm quality and production (Li et al., 2011; Srivastava and Gupta, 2018). Regarding BPA analogues, male rodent studies suggest that BPS and BPF exposure can negatively affect fertility (Kim et al., 2024). BPS has been associated with reduced sperm quality in males (Ullah et al., 2021), and BPF has been shown to impair spermatogenesis, leading to reduced sperm count and motility, which are crucial for male fertility (Fatai and Aribidesi, 2022). However, literature data on human studies are scarce. In a recent systematic review and meta-analysis (Martínez et al., 2023), comprising 32 selected articles, the relationship between various EDCs, including bisphenols measured in urine and sperm quality parameters was assessed. The overall findings revealed significant heterogeneity between studies. Additionally, even less is known about the relationship between urinary exposure to the main analogues of BPA, BPF and BPS, and their potential association with sperm quality (Benson et al., 2021; Chen et al., 2022; Ghayda et al., 2019; Siracusa et al., 2018).

Therefore, the present study aimed to examine the relationship between urinary concentrations of BPA, BPF, and BPS and semen quality parameters among adult Spanish men from the Led-Fertyl study cohort, with the goal of providing novel insights into the potential effects of these commonly encountered chemicals on male reproductive health.

2. Materials and methods

2.1. Study population

This cross-sectional study is based on the Led-Fertyl *"Lifestyle and Environmental Determinants of Seminogram and other Male Fertility-related Parameters"* cohort which enrols wellbeing men. Eligibility criteria, exclusion criteria and recruitment details were described in more details elsewhere (Valle-Hita et al., 2024). Briefly, the exclusion criteria encompassed 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 study included 200 participants, but five participants were excluded for various reasons: i) two had missing values for the main outcomes; ii) two had zero sperm units and their sperm concentration was also zero, and one participant had a sperm vitality equal to zero. Given the undiagnosed pathological nature of these three participants conditions, it was deemed inappropriate to include them in our analysis. Therefore, a total of 195 participants were included in our analysis (Fig. 1). Briefly, the study encompasses wellbeing men aged between 18 and 40 years, drawn from the general population. Participants were recruited between February 2021 and April 2023 using various methods, including video ads on online platforms, flyers and posters in hospitals and healthcare centers, and outreach at the university and public events. Interested individuals were invited to contact the study team, and all participants provided informed consent both online and in writing. Lifestyle, sociodemographic characteristics, and medical data were collected by online questionnaires. Biological samples (urine and semen) were collected during an in-person visit at the Hospital Universitari Sant Joan de Reus (Reus, Tarragona, Spain). All participants provided both online and written informed consent, and the project protocol received approval from the Ethics Committee of the Institut d'Investigacions Sanitàries

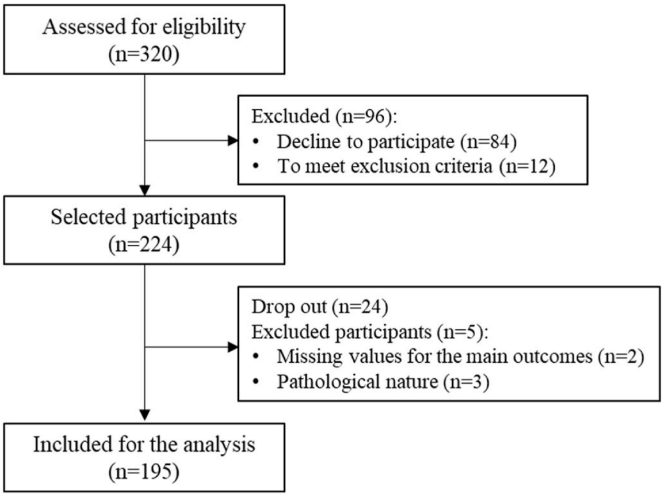

Fig. 1. Flow chart.

Pere i Virgili (Ref. CEIM: 181/2019).

2.2. Biological samples collection

2.2.1. Urine samples

Fasting spot urine samples were aliquoted in polypropylene tubs and were kept at −80 °C until the laboratory analysis. Urinary concentrations of BPA, BPF and BPS were quantified following the methodology previously described by González et al. (2019). In brief, 500 μL of urine sample was added into a glass cryotube. Each sample was first spiked with a mix of internal standards containing Bisphenol A D_{16}, Bisphenol $S^{13}C_{12}$, and Bisphenol $F^{13}C_6$ at 5 ng/mL. Then, 50 μL of enzyme solution (β-glucuronidase/sulfatase) was added to the sample. After that, the mixture was incubated at 37 °C for 24 h, before stopping the hydrolysis reaction at 4 °C. Next, 1.5 mL of acetonitrile was added and allowed to stand at room temperature for 10 min, followed by centrifugation at 3500 rpm for 4 min. Subsequently, 1 mL of the sample was transferred to a clean vial, to which 85 μL of tetrachloroethylene (Sigma-Aldrich (Missouri, USA)) and 100 μL of acetic anhydride (Sigma-Aldrich (Missouri, USA)) were added. In a separate glass tube, 3 mL of deionized water (Milli-Q system from Millipore (Burlington, MA, USA)) and 300 μL of K_2CO_3 (Sigma-Aldrich (Missouri, USA)) were added. Rapidly, the sample was transferred to the glass tube and vortexed. Following centrifugation at 2100 rpm for 4 min, 70 μL of the lower phase was transferred into a 100 μL insert, and 2 μL was eventually injected into the GC-MS system (the analysis was performed using an 8890A GC coupled to a MS/QqQ 7010B from Agilent Technologies, Santa Clara, CA, USA). In addition, it was implemented a comprehensive set of Quality Assurance (QA) and Quality Control (QC) procedures. QA mechanisms included strict adherence to Standard Operating Procedures and the involvement of trained analytical chemistry staff using traceable standards. QC measures involved analyzing blanks with every set of samples, assessing repeatability by analyzing QC samples in triplicate, and verifying accuracy through sample fortifications or spikes.

The targets for analysis, including BPA, BPF and BPS, were separated on an Agilent J&W HP-5MS column (30 m × 0.250 mm x 0.25 μm, J&W Scientific, Folsom, CA, USA) with electron impact ionization (70 eV) in multiple reaction mode. Quality controls including pooled urine samples spiked with the targets and a procedural blank were conducted along with each analysis. The limits of detection (LODs) of BPA, BPF and BPS were 0.13, 0.0093 and 0.0350 ng/mL, respectively. The measured values below the LODs were calculated as ½ LOD (González et al., 2019). The detection rates were 79.5%; 95.0% and 98.5% respectively for BPA, BPS and BPF. The coefficient of variance intra-assay and inter-assay of the quality control sample included in the analysis were measured at 8.64 % and 22.30 % for BPA, at 17.37 % and 18.75 % for BPF, and 8.18 % and 0.52 % for BPS, respectively. The recoveries for BPA, BPS and BPF were 83.7%, 95.3% and 80.7% respectively. Urinary creatinine was measured to adjust for urine dilution based on the Jaffé method (Cobas Pro c503 module (Roche Diagnostics)). Urinary bisphenol concentrations were adjusted for creatinine levels in urine samples to ensure consistent comparisons and streamline the identification of significant impacts.

2.2.2. Semen samples

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 were analysed. 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). 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.

2.3. Assessment of dietary bisphenol intake

The dietary intake of the three bisphenols (BPA, BPS and BPF) was estimated using the methodology previously detailed by Lassen et al. (2013). In summary, we multiplied the urine concentration levels for each bisphenol (measured in ng/mL) by the estimated total 24-h urine volume (mL/day) followed by division by the body weight of our participants (kg).

The European Food Safety Authority (EFSA) has set a TDI specifically for BPA, as previously mentioned. However, specific TDIs for BPA analogues such as BPS and BPF have not been specified (EFSA, 2023). Since all participants exceeded the recently established TDI by the EFSA, the analysis was conducted considering two categories: participants above and below 100 times the TDI (20 ng/kg body weight/day), and their association with sperm quality parameters.

2.4. Statistical analyses

The most updated Led-Fertyl database (from May 2023) was employed for the statistical analysis. The Kolmogorov–Smirnov test was utilized to assess normal distribution. Semen volume, total sperm count, sperm concentration and normal sperm morphology were transformed by square root to approach normality of the residuals. Led-Fertyl study demographic participant characteristics are reported as means ± standard deviations (SD) or median (Pc25–Pc75) for continuous variables and number (%) for categorical variables. Multiple linear regression models were fitted to estimate the associations between urine bisphenols levels and semen quality parameters (semen volume, total sperm count, sperm concentration, sperm vitality, total motility, progressive motility, and normal sperm forms). Bisphenols (BPA, BPS, and BPF) urinary concentrations (in ng/mL) were first standardized by creatinine and then log-transformed. These log-transformed concentrations were subsequently categorized into quartiles, with the lowest quartile serving as the reference category, or analysed as a continuous variable. Results were reported as β-coefficients and their 95% confidence intervals (CI). Models were adjusted for several a priori selected potential confounders (Benson et al., 2021; Ribeiro et al., 2017; Salas-Huetos et al., 2017) including age (years), sexual abstinence (days before delivery of the semen sample), education level (high school or less, college or high education), body mass index (kg/m^2), smoking status (never, current, former), physical activity (MET min/week), adherence to Mediterranean diet score using the MEDAS questionnaire (Schröder et al., 2011) (0–14 points) and season (winter vs summer, spring, or fall).

Logistic regression analyses were conducted to assess the association between log-transformed of creatinine-adjusted concentrations (in ng/mg of creatinine) bisphenols exposure and the likelihood of having semen parameters below the WHO reference values (WHO, 2010). Results were reported as odds ratios (OR) and their 95% confidence intervals (CIs).

Furthermore, sensitivity analyses were conducted by categorizing the estimated exposure to BPA, BPS and BPF (in ng/kg body weight/day) based on a threshold of 100 times the EFSA's recommended TDI for total BPA dietary intake (lower to this threshold or equal/higher to this threshold). The analysis assessed how exposure levels relative to this threshold were associated with semen quality parameters.

The statistical analysis was carried out using Stata 16 (StataCorp) software, and a significance level of 0.05 was used to evaluate the results.

3. Results

A total of 195 men (mean age ± SD: 28.4 ± 5.53 years) were included, in the current analysis. The Led-Fertyl characteristics of the included participants are depicted in Table 1. Most of the participants studied had a MEDAS score higher than 7 out of 14 (80%), were non-smokers (75%) and had normal weight (59%). Only 6% of the population had obesity (it is defined as having a body mass index (BMI) of 30 or higher, which is a measure of body fat based on height and weight), while 12% were identified as current smokers. The median [P25; P75] concentration levels for BPA, BPF and BPS were 0.417 [0.124–0.864] ng/mg of creatinine; 0.119 [0.063–0.240] ng/mg of creatinine and 0.477 [0.265–1.010] ng/mg of creatinine, respectively.

Table 2 shows the multiple-adjusted β-coefficients and their corresponding 95% CIs for seminogram parameters in continuous and across quartiles of urinary log-transformed creatinine-adjusted BPA, BPS and BPF concentrations. Compared with participants in the lowest quartile of urinary BPA and BPF concentrations, those in the highest quartile had a lower percentage of sperm vitality (β: 6.90 %; CI: 11.60 to −2.15 and β: 9.68 %; CI: 14.43 to −4.94; respectively). In addition, when urinary bisphenols concentrations were analysed as a continuous variable, log-transformed of creatinine-adjusted BPA and BPF concentrations (in ng/mg of creatinine) were inversely associated with the percentage of sperm vitality (β: 3.56 %; CI: 6.48 to −0.63 and β: 4.14 %; CI: 6.97 to −1.31; respectively). No other significant associations were found for the other sperm quality parameters.

In addition, the log-transformed concentrations of creatinine-adjusted BPF (in ng/mg of creatinine) were significantly associated with a reduction in the odds of abnormal sperm vitality ((odds ratios (OR) and their 95% confidence intervals (CIs): 0.360, 95% CI: 0.130 to 0.980)). However, for the other sperm parameters (sperm concentration, total sperm count, total motility, progressive motility, and morphology), neither BPF, BPS, nor BPA showed any statistically significant relationship (Figs. 1S–3S, Supplementary Material).

In the current study, all participants largely exceeded (45.45 ng/kg body weight/day) the current EFSA TDI (0.2 ng/kg body weight/day) (EFSA, 2023). Although exposures to the analogues BPS and BPF were below the estimated BPA levels (26.57 and 25.12 ng/kg body weight/day, respectively), they still exceeded the EFSA's TDI. Participants with BPF exposure greater than 100 times the EFSA recommended TDI had a lower percentage of sperm vitality (β: 7.98%; CI: 13.76 to −2.20) compared with those below this threshold (Table 1S, Supplementary Material).

Table 1
Led-Fertyl study participant characteristics.

	Total population n = 195
Demographic characteristics	
Age (years)	28.4 ± 5.57
Educational level	
High school or less	70 (35.9)
College or high education	125 (64.1)
Smoking status	
Never	146 (74.9)
Current	24 (12.3)
Former	25 (12.8)
Physical activity (MET min/week)	3595 [1794–5314]
Waist circumference (cm)	81.8 [77.1–87.6]
BMI (kg/m^2)	24.4 ± 3.19
Mediterranean Diet Adherence Screener –(MEDAS)-score (0–14 points)	8 [7–9]
Semen parameters	
Abstinence time (days)	4 [3–5]
Semen volume (ml)	3.5 [2.5–4.5]
Semen volume <1.5 mL	6 (3.1)
Total sperm count (× 10^6 spz)	168.4 [98.8–283.8]
Total sperm count <39 × 10^6 spz	15 (7.7)
Sperm concentration (× 10^6 spz/ml)	48.6 [29–84.5]
Sperm concentration <15 × 10^6 spz/ml	18 (9.2)
Sperm vitality (%)	80.2 ± 11.5
Vitality <58%	9 (4.62)
Total motility (%)	59.9 ± 16.4
Total motility <40% motile	22 (11.3)
Progressive motility (%)	43.68 ± 16.7
Progressive motility <32% motile	53 (27.2)
Normal sperm morphology (%)	9.5 [5–15]
Sperm morphology <4% normal	26 (13.3)
Semen season collection	
Winter	41 (21.0)
Summer	36 (18.5)
Spring	72 (36.9)
Fall	46 (23.6)
Bisphenol urinary concentrations	
Bisphenol A (ng/mg of creatinine)	0.417 [0.124–0.864]
Bisphenol S (ng/mg of creatinine)	0.477 [0.265–1.010]
Bisphenol F (ng/mg of creatinine)	0.119 [0.063–0.240]

Abbreviations: METS: metabolic equivalent of task; BMI: body mass index. Values are reported as means ± standard deviations (SD) or median [Pc25–Pc75] for continuous variables and number (%) for categorical variables.

4. Discussion

In wellbeing men of reproductive age, we have demonstrated, for the first time, a negative association between urinary concentrations of certain bisphenol chemicals, specifically BPA and BPF, and sperm vitality.

Previous human studies have explored the association between urinary BPA and sperm quality parameters in the general population (Adoamnei et al., 2018; Goldstone et al., 2015; Kim et al., 2024; Meeker et al., 2010; Omran et al., 2018; Pollard et al., 2019). However, the current body of data is both limited and heterogeneous, making it difficult to draw conclusive findings. This highlights a clear gap in high-quality studies analysing the relationship between these chemicals, sperm quality and male fertility (Martínez et al., 2023).

The median urinary concentration of BPA (0.42 ng/mL) reported in the current article was slightly lower than the other previous studies conducted in Spain, the USA, Denmark or China (Adoamnei et al., 2018; Chen et al., 2022; Meeker et al., 2010; Pollard et al., 2019), with a median between 2.8 and 1.3 ng/mL). However, it was quite similar to the concentration reported (0.55 ng/mL) in another study conducted in the USA (Goldstone et al., 2015). So far, only two studies have previously investigated the association between BPA and its analogues, BPS and BPF, in urine, and human sperm quality. One of them involved 556 participants (age range 18–20 years), where no association was reported between urinary bisphenols concentration and sperm quality parameters (Benson et al., 2021). In contrast, in a cohort of the Chinese men from an infertility clinic including 984 participants (Chen et al., 2022), higher urinary BPA concentrations were associated with increased odds ratios (ORs) of having below normality reference sperm concentration, total sperm count, progressive motility, and total motility. In addition, in the aforementioned study, BPS exposure was associated with increased ORs for having below reference progressive motility and total motility. These discrepancies between studies may be explained by the type of participants, general vs clinical population, included in the analysis of Benson et al. (2021); Chen et al. (2022), respectively; but also, by the level of exposure to these chemicals between populations. Specifically,

Table 2
Multiple-adjusted β-coefficients and its 95% CI for seminogram parameters across urinary log-transformed creatinine-adjusted BPA, BPS and BPF concentrations.

n = 195	BPA		BPS		BPF	
	Quartiles (Log-transformed creatinine-adjusted concentration (ng/mg))	**β-coefficients (95%CI)**	Quartiles (Log-transformed creatinine-adjusted concentration (ng/mg))	**β-coefficients (95%CI)**	Quartiles (Log-transformed creatinine-adjusted concentration (ng/mg))	**β-coefficients (95%CI)**
Semen volume (mL) [a]	Q1 (<0.124)	Reference	Q1 (<0.265)	Reference	Q1 (<0.063)	Reference
	Q2 (0.124–0.417)	0.06 (−0.09 to 0.21)	Q2 (0.265–0.477)	−0.78 (−0.23 to 0.76)	Q2 (0.063–0.119)	0.06 (−0.10 to 0.21)
	Q3 (0.417–0.864)	0.03 (−0.12 to 0.19)	Q3 (0.477–1.010)	**−0.20 (-0.35 to -0.49)**	Q3 (0.119–0.240)	−0.11 (−0.26 to 0.05)
	Q4 (>0.864)	−0.07 (−0.23 to 0.08)	Q4 (>1.010)	−0.09 (−0.25 to 0.58)	Q4 (>0.240)	−0.07 (−0.26 to 0.54)
	Continuous (1.73 ± 14.0)	−0.59 (−0.15 to 0.38)	Continuous (0.99 ± 2.03)	−0.08 (−0.18 to 0.02)	Continuous (0.97 ± 2.03)	−0.07 (−0.17 to 0.18)
Total sperm count (× 10^6) [a]	Q1 (<0.124)	Reference	Q1 (<0.265)	Reference	Q1 (<0.063)	Reference
	Q2 (0.124–0.417)	0.26 (−1.98 to 2.51)	Q2 (0.265–0.477)	0.25 (−1.93 to 2.46)	Q2 (0.063–0.119)	0.53 (−1.70 to 2.77)
	Q3 (0.417–0.864)	−0.01 (−2.25 to 2.54)	Q3 (0.477–1.010)	−1.17 (−3.39 to 1.03)	Q3 (0.119–0.240)	0.52 (−1.77 to 2.81)
	Q4 (>0.864)	1.2 (−1.08 to 3.48)	Q4 (>1.010)	0.77 (−1.45 to 2.99)	Q4 (>0.240)	0.96 (−1.35 to 3.29)
	Continuous (1.73 ± 14.0)	0.64 (−0.76 to 2.01)	Continuous (0.99 ± 2.03)	0.07 (−1.44 to 1.59)	Continuous (0.97 ± 2.03)	0.07 (−1.28 to 1.42)
Sperm concentration (× 10^6/mL) [a]	Q1 (<0.124)	Reference	Q1 (<0.265)	Reference	Q1 (<0.063)	Reference
	Q2 (0.124–0.417)	−0.25 (−1.26 to 1.21)	Q2 (0.265–0.477)	0.60 (−0.61 to 1.82)	Q2 (0.063–0.119)	0.01 (−1.22 to 1.24)
	Q3 (0.417–0.864)	0.15 (−1.08 to 1.39)	Q3 (0.477–1.010)	0.07 (−1.14 to 1.29)	Q3 (0.119–0.240)	0.45 (−0.80 to 1.72)
	Q4 (>0.864)	0.86 (−0.39 to 2.11)	Q4 (>1.010)	1.01 (−0.21 to 2.23)	Q4 (>0.240)	0.48 (−0.79 to 1.76)
	Continuous (1.73 ± 14.0)	0.63 (−0.12 to 1.40)	Continuous (0.99 ± 2.03)	0.39 (−0.43 to 1.23)	Continuous (0.97 ± 2.03)	0.23 (−0.54 to 0.95)
Sperm vitality (%)	Q1 (<0.124)	Reference	Q1 (<0.265)	Reference	Q1 (<0.063)	Reference
	Q2 (0.124–0.417)	**−5.85 (-10.53 to -1.18)**	Q2 (0.265–0.477)	2.13 (−2.41 to 7.05)	Q2 (0.063–0.119)	**−5.53 (-10.11 to -0.96)**
	Q3 (0.417–0.864)	**−7.01 (-11.69 to -2.34)**	Q3 (0.477–1.010)	−3.74 (−3.22 to 5.71)	Q3 (0.119–0.240)	−4.00 (−8.61 to 0.76)
	Q4 (>0.864)	**−6.90 (-11.60 to -2.15)**	Q4 (>1.010)	3.51 (−1.23 to 8.27)	Q4 (>0.240)	**−9.68 (-14.43 to -4.94)**
	Continuous (1.73 ± 14.0)	**−3.56 (-6.48 to -0.63)**	Continuous (0.99 ± 2.03)	1.67 (−1.55 to 4.91)	Continuous (0.97 ± 2.03)	**−4.14 (-6.97 to -1.31)**
Total motility (%)	Q1 (<0.124)	Reference	Q1 (<0.265)	Reference	Q1 (<0.063)	Reference
	Q2 (0.124–0.417)	1.78 (−5.00 to 8.56)	Q2 (0.265–0.477)	1.93 (−4.74 to 8.60)	Q2 (0.063–0.119)	−1.29 (−7.96 to 5.38)
	Q3 (0.417–0.864)	1.32 (−5.46 to 8.11)	Q3 (0.477–1.010)	−3.33 (−10.0 to 3.32)	Q3 (0.119–0.240)	4.89 (−1.95 to 11.7)
	Q4 (>0.864)	1.53 (−5.30 to 8.36)	Q4 (>1.010)	−0.50 (−7.19 to 621)	Q4 (>0.240)	2.04 (−4.87 to 8.97)
	Continuous (1.73 ± 14.0)	1.64 (−2.53 to 5.58)	Continuous (0.99 ± 2.03)	−1.66 (−6.24 to 2.87)	Continuous (0.97 ± 2.03)	1.34 (−2.72 to 5.41)
Progressive motility (%)	Q1 (<0.124)	Reference	Q1 (<0.265)	Reference	Q1 (<0.063)	Reference
	Q2 (0.124–0.417)	4.00 (−2.95 to 10.96)	Q2 (0.265–0.477)	0.44 (−6.45 to 7.34)	Q2 (0.063–0.119)	−0.56 (−6.62 to 7.34)
	Q3 (0.417–0.864)	3.56 (−3.44 to 10.51)	Q3 (0.477–1.010)	−2.66 (−9.55 to 4.23)	Q3 (0.119–0.240)	0.63 (−0.89 to 7.70)
	Q4 (>0.864)	3.67 (−3.39 to 10.73)	Q4 (>1.010)	0.15 (−6.78 to 7.08)	Q4 (>0.240)	0.58 (−6.53 to 7.70)
	Continuous (1.73 ± 14.0)	2.93 (−1.36 to 7.22)	Continuous (0.99 ± 2.03)	−0.18 (−4.89 to 4.51)	Continuous (0.97 ± 2.03)	0.40 (−3.79 to 4.60)
Normal sperm morphology (%)[a]	Q1 (<0.124)	Reference	Q1 (<0.265)	Reference	Q1 (<0.063)	Reference
	Q2 (0.124–0.417)	−0.02 (−0.47 to 0.47)	Q2 (0.265–0.477)	−0.09 (−0.56 to 0.37)	Q2 (0.063–0.119)	0.18 (−0.28 to 0.61)
	Q3 (0.417–0.864)	0.02 (−0.44 to 0.50)	Q3 (0.477–1.010)	0.92 (−0.37 to 0.56)	Q3 (0.119–0.240)	0.23 (−0.24 to 0.71)
	Q4 (>0.864)	−0.30 (−0.78 to 0.17)	Q4 (>1.010)	0.22 (−0.24 to 0.69)	Q4 (>0.240)	−0.09 (−0.58 to 0.39)
	Continuous (1.73 ± 14.0)	−0.08 (−0.37 to 0.21)	Continuous (0.99 ± 2.03)	0.13 (−0.18 to 0.45)	Continuous (0.97 ± 2.03)	−0.06 (−0.34 to 0.22)

Estimates marked **in bold** indicate $p < 0.05$.

Abbreviations: BPA: Bisphenol A; BPF: Bisphenol F and BPS: Bisphenol S.

Multiple linear regression (MLR) models were fitted to assess the β-coefficients, and its 95% CI for sperm parameters across urinary log-transformed creatinine-adjusted BPA, BPS and BPF concentrations. MLR models were adjusted by age (years), sexual abstinence (days before delivery of the semen sample), education level (High school or less, College or high education), body mass index (kg/m^2), smoking status (never, current, former), physical activity (MET min/week), adherence to Mediterranean diet MEDAS score (0–14 points) and season (winter vs spring, summer, or fall).

Q1-Q4 = Quartiles of Creatinine-Adjusted Urinary Bisphenol A, S, and F concentrations among Led-Fertyl participants (in ng/mg of creatinine). Values are reported as means ± standard deviations for the continuous form (in ng/mg of creatinine).

[a] Semen volume, total sperm count, sperm concentration and normal sperm morphology were transformed by square root to approach normality of the residuals.

Benson et al., 2021 reported 1.3 ng/mL for BPA, 0.06 ng/mL for BPS and 0.14 ng/mL for BPF, whereas Chen et al. (2022) reported concentrations of 2.24 ng/mL for BPA, 0.35 ng/mL for BPS and 0.77 ng/mL for BPF. Moreover, the median urinary concentration of BPA in our participants (0.417 ng/mL) was similar to that reported by Benson et al. (2021). For BPS and BPF analogues, our study medians (0.60 ng/mL and 0.14 ng/mL, respectively) were consistent with those reported by Chen et al. (2022).

Studies in male rats exposed to BPA have shown that they exhibit impaired reproductive function, characterized by reduced testosterone levels, impaired Sertoli cell function, and disrupted sperm quality and production (Chen et al., 2022; Jin et al., 2013; Li et al., 2011; Srivastava and Gupta, 2018); these findings establish BPA as a genuine reproductive toxic. Although there is less scientific evidence is available regarding BPS and BPF analogues in animals, recent articles suggest that these chemicals may act very similarly to BPA, or may even have greater endocrine-disrupting potential (Ullah et al., 2019a, 2019b). Regarding *in vitro* studies, some authors have shown that the BPS and BPF analogues may have higher endocrine-disrupting activity than BPA, suggesting that replacing BPA with these analogues may not be safe (Martínez et al., 2020; Ullah et al., 2019b).

Bisphenols are well-known EDCs, that can interfere with the hormonal system, including the regulation of hormones involved in sperm production. In addition, they can induce oxidative stress in germinal cells leading to DNA damage or altering the spermatogenesis cycle (Meeker et al., 2010; Omran et al., 2018; Ullah et al., 2019b; Wu et al., 2017). These mechanisms may ultimately contribute to the disturbance of certain sperm parameters, such as sperm vitality. In addition, there is growing evidence that exposure to certain EDCs, such as the bisphenol family, particularly during critical periods like early development and reproductive age, is a major concern. These harmful effects not only impact the individual but may also be transmitted to the next generation (Li et al., 2014). Therefore, it is crucial to identify and address all potential contributing factors (Barratt et al., 2018; Diamanti-Kandarakis et al., 2009; Lokeshwar et al., 2021).

Currently, the EFSA has set the TDI for BPA at 0.2 ng/kg body weight/day (EFSA, 2023). This is a reduction of 20,000 times compared to the previous TDI of 4 μg/kg body weight/day from 2015 (EFSA, 2015). This enormous reduction underscores the imperative to continue generating scientific evidence about these chemicals. Our results revealed that all participants were above the new TDI limits established by the EFSA. Furthermore, the estimated exposure to analogues (BPS and BPF) exceeded this threshold. However, as previously mentioned, there is no specific TDI established for these compounds (EFSA, 2023). Therefore, it is essential to incorporate BPS and BPF into research studies to ascertain whether they serve as safe alternatives or not.

The current study presents some limitations and strengths, which deserve to be mentioned. Firstly, owing to the inherent nature of observational studies, cause-effect relationships cannot be driven by observational studies, as they are primarily designed to identify associations and patterns rather than causal links. Secondly, due to the cross-sectional design, we were limited to obtaining only a single urine and semen sample per participant. While fasting urine samples may not fully capture dietary exposure throughout the day, they offer a useful alternative and less invasive measure in large-scale epidemiologic studies where 24-h urine collection is difficult and not feasible. Considering the spermatogenic cycle's duration of approximately 90 days, the measured urine levels of bisphenols might have been captured outside critical time frames. However, these urine and semen samples were collected on the same day, representing an ordinary day within the participant's regular routine. Nevertheless, there is currently insufficient information regarding the specific number of urine samples necessary to obtain a reliable estimate of BPA biomarkers for adults over a day or longer (Morgan et al., 2018). Moreover, it should be noted that evaluating the dietary intake of bisphenols based on concentration levels derived from fasting spot urine may pose a limitation. However, it is noteworthy that our estimated daily exposures align in magnitude with those reported in studies conducted on male populations in Denmark (Lassen et al., 2013) and the USA (Lakind and Naiman, 2011; LaKind and Naiman, 2008). Thirdly, while we have accounted for numerous potential confounders in our models, it is important to acknowledge that residual confounding may still exist. As a strength, it is noteworthy that the current study collected data from a wellbeing general population, with participants not sourced from an infertility clinic. Although, we cannot ensure that they are fertile. It's important to highlight that the detection limits for each bisphenol were notably consistent and slightly lower compared to recent studies (Benson et al., 2021; Chen et al., 2022) mitigating the risk of overestimating the results. Additionally, the high level of standardization in the study's exposure and outcome measurements, processed through the CASA SCA® system, minimized potential subjectivity. Nonetheless, we acknowledge that with a relatively small sample size, there is always some degree of uncertainty and have taken care to interpret the results with appropriate caution.

These findings support the growing body of evidence linking the potential effect that bisphenols exposure may have on male fertility as observed in animal models and some human studies. Our findings could be used as a basis for generating new hypotheses and designing subsequent studies. This includes addressing unanswered questions about the mechanisms underlying the observed associations and considering the role of various factors that might influence bisphenol exposure and its effects on reproductive health. We will emphasize the need for longitudinal studies to better understand the long-term effects of BPA and its substitutes. Additionally, we will suggest the importance of considering both urinary and dietary exposure assessments when evaluating semen quality. This could involve conducting studies that use total dietary assessments and 24-h urine collections to obtain results that more closely reflect real-world scenarios.

5. Conclusions

In conclusion, in male well-being participants, we demonstrate an inverse association between urine levels of BPF and BPA and sperm vitality. In addition, exposure estimations of BPF further reinforce the main association previously mentioned. Given the significant implications that these functional abnormalities could entail, additional studies replicating these results and assessing potential associations between these EDCs and sperm quality and male infertility are crucial.

Ethics approval and consent to participate

Every participant provided informed consent through both online and written means, and the project protocol received approval from the ethical committee of Institut d'Investigacions Sanitàries Pere i Virgili (Ref. CEIM: 181/2019).

Funding statement

MÁ.M is supported by the Sara Borrell postdoctoral fellowship [CD21/00045- Instituto de Salud Carlos III (ISCIII)] and the M-AES mobility grant [MV23/00006-Instituto de Salud Carlos III (ISCIII)]. This work was supported by the Spanish government's official funding agency for biomedical research, ISCIII, through the Fondo de Investigación para la Salud (FIS) and co-funded by European Union ERDF/ESF, "A way to make Europe"/"Investing in your future" [PI21/01447], and the support partially of the Diputació de Tarragona (2021/11-No. Exp. 8004330008-2021-0022,642). JSS, senior autor of this article, gratefully acknowledges the financial support by ICREA under the ICREA Academia program. MFdlP was supported by a predoctoral grant from the Rovira i Virgili University and Diputació de Tarragona (2020-PMF-PIPF-8). C.V.-H. was supported by a predoctoral grant from the Generalitat de Catalunya (2022 FI_B100108). E.D.-C has received a Contrato Pre-doctoral de Formación en Investigación en Salud (PFIS FI22/00018) of the Acción Estratégica en Salud program (AES) from the Carlos III Health Institute (ISCIII), Spanish Ministry of Health.

Data sharing statement

The datasets generated and analysed in the current study are not publicly accessible due to data regulations and ethical constraints. This measure has been implemented to ensure the consent of research participants, whose original agreement only permitted the use of their data by the original research team. However, collaborative data analysis may be feasible upon submission of a formal request via a letter addressed to the corresponding author.

CRediT authorship contribution statement

María Ángeles Martínez: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Albert Salas-Huetos:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization. **María Fernández de la Puente:** Writing – review & editing, Validation, Methodology, Investigation. **Cristina Valle-Hita:** Writing – original draft, Validation, Methodology, Investigation. **Montse Marquès:** Writing – original draft, Validation, Methodology, Investigation. **Claudia Del Egido-González:** Writing – review & editing, Validation, Methodology, Investigation. **Estefanía Davila-Cordova:** Writing – review & editing, Validation, Methodology, Investigation. **Cristina Mestres:** Writing – review & editing. **Maria Skaalum Petersen:** Writing – review & editing, Validation, Supervision, Investigation. **Nancy Babio:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition, Conceptualization. **Jordi Salas-Salvadó:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization.

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.

Acknowledgments

We extend our gratitude to the participants of the Led-Fertyl study for their enthusiastic collaboration, and we express our appreciation to the dedicated Led-Fertyl staff for their exceptional support. We would also like to thank the Spanish government's official funding agency for biomedical research, Instituto de Salud Carlos III (ISCIII). Finally, we would like also to extend special acknowledgment to the IISPV Biobank (PT20/00197), which is part of the ISCIII Platform for Biobanks and Biomodels, and to Dr. Jesús Garcia Gavilán for his invaluable contribution and expertise in statistical analysis.

Abbreviations

EDCs	Endocrine-disrupting chemical
EFSA	European Food Safety Authority
TDI	tolerable daily intake
BPA	Bisphenol A
BPS	Bisphenol S
BPF	Bisphenol F

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.120086.

References

Adoamnei, E., Mendiola, J., Vela-Soria, F., Fernández, M.F., Olea, N., Jørgensen, N., Swan, S.H., Torres-Cantero, A.M., 2018. Urinary bisphenol A concentrations are associated with reproductive parameters in young men. Environ. Res. 161, 122–128. https://doi.org/10.1016/j.envres.2017.11.002.

Agarwal, A., Baskaran, S., Parekh, N., Cho, C.L., Henkel, R., Vij, S., Arafa, M., Panner Selvam, M.K., Shah, R., 2021. Male infertility. Lancet. https://doi.org/10.1016/S0140-6736(20)32667-2.

Barratt, C.L.R., De Jonge, C.J., Sharpe, R.M., 2018. "Man Up": the importance and strategy for placing male reproductive health centre stage in the political and research agenda. Hum. Reprod. 33, 541–545. https://doi.org/10.1093/humrep/dey020.

Benson, T.E., Gaml-Sørensen, A., Ernst, A., Brix, N., Hougaard, K.S., Hærvig, K.K., Bonde, J.P.E., Tøttenborg, S.S., Lindh, C.H., Ramlau-Hansen, C.H., Toft, G., 2021. Urinary bisphenol a, f and s levels and semen quality in young adult Danish men. Int. J. Environ. Res. Publ. Health 18, 1–12. https://doi.org/10.3390/ijerph18041742.

Calafat, A.M., Ye, X., Wong, L.Y., Reidy, J.A., Needham, L.L., 2008. Exposure of the U.S. Population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environ. Health Perspect. 116, 39–44. https://doi.org/10.1289/ehp.10753.

Chen, P.P., Liu, C., Zhang, M., Miao, Y., Cui, F.P., Deng, Y.L., Luo, Q., Zeng, J.Y., Shi, T., Lu, T.T., Yin, W.J., Lu, W.Q., Yi, G.L., Qiu, G.K., Zeng, Q., 2022. Associations between urinary bisphenol A and its analogues and semen quality: a cross-sectional study among Chinese men from an infertility clinic. Environ. Int. 161. https://doi.org/10.1016/j.envint.2022.107132.

Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr. Rev. https://doi.org/10.1210/er.2009-0002.

EFSA, 2004. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to 2,2-bis(4-hydroxyphenyl)propane bis(2,3-epoxypropyl)ether (bisphenol a diglycidyl ether, BADGE). EFSA J. 2. https://doi.org/10.2903/j.efsa.2004.86.

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 13. https://doi.org/10.2903/j.efsa.2015.3978.

EFSA, 2023. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 21. https://doi.org/10.2903/j.efsa.2023.6857.

Fatai, O.A., Aribidesi, O.L., 2022. Effect of bisphenol F on sexual performance and quality of offspring in Male Wistar rats. Ecotoxicol. Environ. Saf. 244. https://doi.org/10.1016/j.ecoenv.2022.114079.

Ghayda, R.A., Williams, P.L., Chavarro, J.E., Ford, J.B., Souter, I., Calafat, A.M., Hauser, R., Mínguez-Alarcón, L., 2019. Urinary bisphenol S concentrations: potential predictors of and associations with semen quality parameters among men attending a fertility center. Environ. Int. 131. https://doi.org/10.1016/j.envint.2019.105050.

Gimeno, P., Spinau, C., Lassu, N., Maggio, A.F., Brenier, C., Lempereur, L., 2015. Identification and quantification of bisphenol A and bisphenol B in polyvinylchloride and polycarbonate medical devices by gas chromatography with mass spectrometry. J. Separ. Sci. 38, 3727–3734. https://doi.org/10.1002/jssc.201500552.

Goldstone, A.E., Chen, Z., Perry, M.J., Kannan, K., Louis, G.M.B., 2015. Urinary bisphenol A and semen quality, the LIFE study. Reprod. Toxicol. 51, 7–13. https://doi.org/10.1016/j.reprotox.2014.11.003.

González, N., Cunha, S.C., Monteiro, C., Fernandes, J.O., Marquès, M., Domingo, J.L., Nadal, M., 2019. Quantification of eight bisphenol analogues in blood and urine samples of workers in a hazardous waste incinerator. Environ. Res. 176. https://doi.org/10.1016/j.envres.2019.108576.

Jin, P., Wang, X., Chang, F., Bai, Y., Li, Y., Zhou, R., Chen, L., 2013. Low dose bisphenol A impairs spermatogenesis by suppressing eproductive hormone production and promoting germ cell apoptosis in adult rats. J Biomed Res 27, 135–144. https://doi.org/10.7555/JBR.27.20120076.

Kim, S.H., Shin, S.H., Kim, S.M., Jung, S.E., Shin, B.J., Ahn, J.S., Lim, K.T., Kim, D.H., Lee, K., Ryu, B.Y., 2024. Bisphenol analogs downregulate the self-renewal potential of spermatogonial stem cells. World Journal of Men's Health 42. https://doi.org/10.5534/wjmh.230166.

Koch, H.M., Kolossa-Gehring, M., Schröter-Kermani, C., Angerer, J., Brüning, T., 2012. Bisphenol A in 24 h urine and plasma samples of the German Environmental Specimen Bank from 1995 to 2009: a retrospective exposure evaluation. J. Expo. Sci. Environ. Epidemiol. 22, 610–616. https://doi.org/10.1038/jes.2012.39.

Lakind, J.S., Naiman, D.Q., 2011. Daily intake of bisphenol A and potential sources of exposure: 2005-2006 national health and nutrition examination survey. J. Expo. Sci. Environ. Epidemiol. 21, 272–279. https://doi.org/10.1038/jes.2010.9.

LaKind, J.S., Naiman, D.Q., 2008. Bisphenol A (BPA) daily intakes in the United States: estimates from the 2003-2004 NHANES urinary BPA data. J. Expo. Sci. Environ. Epidemiol. 18, 608–615. https://doi.org/10.1038/jes.2008.20.

Lassen, T.H., Frederiksen, H., Jensen, T.K., Petersen, J.H., Main, K.M., Skakkebæk, N.E., Jørgensen, N., Kranich, S.K., Andersson, A.M., 2013. Temporal variability in urinary excretion of bisphenol A and seven other phenols in spot, morning, and 24-h urine samples. Environ. Res. 126, 164–170. https://doi.org/10.1016/j.envres.2013.07.001.

Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Jolles, M., Pinotti, R., Swan, S.H., 2023. Temporal trends in sperm count: a systematic review and meta-regression analysis of samples collected globally in the 20th and 21st centuries. Hum. Reprod. Update 29, 157–176. https://doi.org/10.1093/humupd/dmac035.

Li, D.K., Zhou, Z., Miao, M., He, Y., Wang, J., Ferber, J., Herrinton, L.J., Gao, E., Yuan, W., 2011. Urine bisphenol-A (BPA) level in relation to semen quality. Fertil. Steril. 95, 625–630.e4. https://doi.org/10.1016/J.FERTNSTERT.2010.09.026.

Li, G., Chang, H., Xia, W., Mao, Z., Li, Y., Xu, S., 2014. F0 maternal BPA exposure induced glucose intolerance of F2 generation through DNA methylation change in Gck. Toxicol. Lett. 228, 192–199. https://doi.org/10.1016/J.TOXLET.2014.04.012.

Lokeshwar, S.D., Patel, P., Fantus, R.J., Halpern, J., Chang, C., Kargi, A.Y., Ramasamy, R., 2021. Decline in serum testosterone levels among adolescent and young adult men in the USA. Eur Urol Focus 7, 886–889. https://doi.org/10.1016/j.euf.2020.02.006.

Ma, Y., Liu, H., Wu, J., Yuan, L., Wang, Y., Du, X., Wang, R., Marwa, P.W., Petlulu, P., Chen, X., Zhang, H., 2019. The adverse health effects of bisphenol A and related toxicity mechanisms. Environ. Res. 176, 108575. https://doi.org/10.1016/J.ENVRES.2019.108575.

Martínez, M.Á., Blanco, J., Rovira, J., Kumar, V., Domingo, J.L., Schuhmacher, M., 2020. Bisphenol A analogues (BPS and BPF) present a greater obesogenic capacity in 3T3-L1 cell line. Food Chem. Toxicol. 140. https://doi.org/10.1016/j.fct.2020.111298.

Martínez, M.Á., González, N., Martí, A., Marquès, M., Rovira, J., Kumar, V., Nadal, M., 2021. Human biomonitoring of bisphenol A along pregnancy: an exposure reconstruction of the EXHES-Spain cohort. Environ. Res. 196, 110941. https://doi.org/10.1016/J.ENVRES.2021.110941.

Martínez, M.Á., Marquès, M., Salas-Huetos, A., Babio, N., Domingo, J.L., Salas-Salvadó, J., 2023. Lack of association between endocrine disrupting chemicals and male fertility: a systematic review and meta-analysis. Environ. Res. 217, 114942. https://doi.org/10.1016/J.ENVRES.2022.114942.

Martínez, M.A., Rovira, J., Prasad Sharma, R., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: a Catalonia (Spain) case study. Environ. Res. 166. https://doi.org/10.1016/j.envres.2018.05.008.

Matuszczak, E., Komarowska, M.D., Debek, W., Hermanowicz, A., 2019. The impact of bisphenol A on fertility, reproductive system, and development: a review of the literature. Internet J. Endocrinol. https://doi.org/10.1155/2019/4068717.

Meeker, J.D., Ehrlich, S., Toth, T.L., Wright, D.L., Calafat, A.M., Trisini, A.T., Ye, X., Hauser, R., 2010. Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. Reprod. Toxicol. 30, 532–539. https://doi.org/10.1016/j.reprotox.2010.07.005.

Morgan, M.K., Nash, M., Barr, D.B., Starr, J.M., Scott Clifton, M., Sobus, J.R., 2018. Distribution, variability, and predictors of urinary bisphenol A levels in 50 North Carolina adults over a six-week monitoring period. Environ. Int. 112, 85–99. https://doi.org/10.1016/j.envint.2017.12.014.

Ni, M., Deepika, D., Li, X., Xiong, W., Zhang, L., Chen, J., Kumar, V., 2024. IVIVE-PBPK based new approach methodology for addressing early life toxicity induced by Bisphenol A. Environ. Res. 240, 117343. https://doi.org/10.1016/J.ENVRES.2023.117343.

Ni, M., Li, X., Zhang, L., Kumar, V., Chen, J., 2022. Bibliometric analysis of the toxicity of bisphenol A. Int. J. Environ. Res. Publ. Health. https://doi.org/10.3390/ijerph19137886.

Omran, G.A., Gaber, H.D., Mostafa, N.A.M., Abdel-Gaber, R.M., Salah, E.A., 2018. Potential hazards of bisphenol A exposure to semen quality and sperm DNA integrity among infertile men. Reprod. Toxicol. 81, 188–195. https://doi.org/10.1016/j.reprotox.2018.08.010.

Pollard, S.H., Cox, K.J., Blackburn, B.E., Wilkins, D.G., Carrell, D.T., Stanford, J.B., Porucznik, C.A., 2019. Male exposure to bisphenol A (BPA) and semen quality in the home observation of periconceptional exposures (HOPE) cohort. Reprod. Toxicol. 90, 82–87. https://doi.org/10.1016/j.reprotox.2019.08.014.

Ribeiro, E., Ladeira, C., Viegas, S., 2017. Occupational exposure to Bisphenol A (BPA): a reality that still needs to be unveiled. Toxics. https://doi.org/10.3390/toxics5030022.

Sabuz Vidal, O., Deepika, D., Schuhmacher, M., Kumar, V., 2021. EDC-induced mechanisms of immunotoxicity: a systematic review. Crit. Rev. Toxicol. https://doi.org/10.1080/10408444.2021.2009438.

Salas-Huetos, A., Bulló, M., Salas-Salvadó, J., 2017. Dietary patterns, foods and nutrients in male fertility parameters and fecundability: a systematic review of observational studies. Hum. Reprod. Update 23, 371–389. https://doi.org/10.1093/humupd/dmx006.

Schröder, H., Fitó, M., Estruch, R., Martínez-González, M.A., Corella, D., Salas-Salvadó, J., Lamuela-Raventós, R., Ros, E., Salaverría, I., Fiol, M., Lapetra, J., Vinyoles, E., Gómez-Gracia, E., Lahoz, C., Serra-Majem, L., Pintó, X., Ruiz-Gutierrez, V., Covas, M.I., 2011. A Short screener is valid for assessing mediterranean diet adherence among older Spanish men and women. J. Nutr. 141, 1140–1145. https://doi.org/10.3945/jn.110.135566.

Siracusa, J.S., Yin, L., Measel, E., Liang, S., Yu, X., 2018. Effects of bisphenol A and its analogs on reproductive health: a mini review. Reprod. Toxicol. https://doi.org/10.1016/j.reprotox.2018.06.005.

Srivastava, S., Gupta, P., 2018. Alteration in apoptotic rate of testicular cells and sperms following administration of Bisphenol A (BPA) in Wistar albino rats. Environ. Sci. Pollut. Control Ser. 25, 21635–21643. https://doi.org/10.1007/s11356-018-2229-2.

Thomas Zoeller, R., Brown, T.R., Doan, L.L., Gore, A.C., Skakkebaek, N.E., Soto, A.M., Woodruff, T.J., Vom Saal, F.S., 2012. Endocrine-disrupting chemicals and public health protection: a statement of principles from the Endocrine Society. Endocrinology 153, 4097–4110. https://doi.org/10.1210/en.2012-1422.

Ullah, A., Pirzada, M., Afsar, T., Razak, S., Almajwal, A., Jahan, S., 2019a. Effect of bisphenol F, an analog of bisphenol A, on the reproductive functions of male rats. Environ. Health Prev. Med. 24. https://doi.org/10.1186/s12199-019-0797-5.

Ullah, A., Pirzada, M., Jahan, S., Ullah, H., Khan, M.J., 2019b. Bisphenol A analogues bisphenol B, bisphenol F, and bisphenol S induce oxidative stress, disrupt daily sperm production, and damage DNA in rat spermatozoa: a comparative in vitro and in vivo study. Toxicol. Ind. Health 35, 294–303. https://doi.org/10.1177/0748233719831528.

Ullah, H., Ullah, F., Rehman, O., Jahan, S., Afsar, T., Al-Disi, D., Almajwal, A., Razak, S., 2021. Chronic exposure of bisphenol S (BPS) affect hypothalamic-pituitary-testicular activities in adult male rats: possible in estrogenic mode of action. Environ. Health Prev. Med. 26. https://doi.org/10.1186/s12199-021-00954-0.

Valle-Hita, C., Salas-Huetos, A., de la Puente, M.F., Martínez, M.Á., Canudas, S., Palau-Galindo, A., Mestres, C., Manzanares, J.M., Murphy, M.M., Marquès, M., Salas-Salvadó, J., Babio, N., 2024. Ultra-processed food consumption and semen quality parameters in the Led-Fertyl study. Hum Reprod Open 2024. https://doi.org/10.1093/hropen/hoae001.

Vander Borght, M., Wyns, C., 2018. Fertility and infertility: definition and epidemiology. Clin. Biochem. https://doi.org/10.1016/j.clinbiochem.2018.03.012.

Völkel, W., Colnot, T., Csanády, G.A., Filser, J.G., Dekant, W., 2002. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. Chem. Res. Toxicol. 15, 1281–1287. https://doi.org/10.1021/tx025548t.

WHO, 2021. WHO Laboratory Manual for the Examination and Processing of Human Semen, sixth ed.

WHO, 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen. World Health Organization.

Wu, H., Estill, M.S., Shershebnev, A., Suvorov, A., Krawetz, S.A., Whitcomb, B.W., Dinnie, H., Rahil, T., Sites, C.K., Pilsner, J.R., 2017. Preconception urinary phthalate concentrations and sperm DNA methylation profiles among men undergoing IVF treatment: a cross-sectional study. Hum. Reprod. 32, 2159–2169. https://doi.org/10.1093/humrep/dex283.