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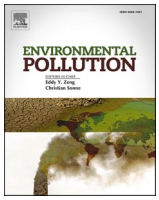

Cross sectional study on exposure to BPA and its analogues and semen parameters in Czech men☆

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ABSTRACT

Exposure to bisphenols has been found to have adverse effects on male reproductive function in animals. Human exposure to bisphenols is widespread. Bisphenol A (BPA) and its analogues, including bisphenol S (BPS), bisphenol F (BPF), and bisphenol AF (BPAF) are utilized in various consumer products such as food contact materials and dental resins. The effects of these compounds on male fertility and spermatogenesis are unclear and findings from human studies are inconsistent. In this cross-sectional study, we evaluated the influence of BPA, BPS, BPF, BPAF (BPs) measured in semen on number of spermatozoa, total motility, progressive motility, morphology, and DNA fragmentation. We also examined the association of bisphenols (BPs) exposure with patients' occupation. A total of 358 patients aged 17–62 years with BMI 18–42 were included in the study from 2019 to 2021. BPs were extracted using solvent extraction followed by preconcentration step and determined by high-performance liquid chromatography and tandem mass spectrometry (LC/MSMS). Bisphenols were detected in 343 from 349 analysed samples (98.3% of all the samples). In 6 samples, the concentration of all BPs was under the limit of detection and in 20 samples under the limit of quantification. We did not find a statistically significant relationship between occupation and BPs. However, we observed significant correlations between the concentration of BPA and a lower motility and normal morphology. For BPS, a significant correlation with a lower ejaculate volume and a lower total sperm count was found. BPF and BPAF were detected only in 14.3% and 23.9% of samples, respectively. For BPF and BPAF, no significant correlations with spermiogram parameters were observed. Our results show that BPs are widespread in the male population (more than 90% of analysed samples), independently of an occupation and in case of BPA and BPS having a negative impact on spermiogram parameters.

1. Introduction

Bisphenols are a group of chemicals used in production of plastics, epoxy resins and other products since the 1960s. Currently, bisphenol A (BPA) is one of the most produced chemicals, with approximately 3.4 million tons produced worldwide annually for manufacture of polycarbonate plastics. (Peretz et al., 2014; Vandenberg et al., 2007; Vandenberg et al., 2009). Due to its resistance, flexibility and durability, BPA-based plastics are widely used as PC plastics, in protective equipment (helmets), medical materials, including dental fillings and seals. They are also widely used in the food industry to produce packaging for durable foods and beverages and are also an important component of

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protective layers covering the inner surface of cans (Peretz et al., 2014; Vandenberg et al., 2007; Vandenberg et al., 2009). Bisphenol A is truly ubiquitous and is also used in a wide range of other commonly used items such as: refrigerators, baby bottles, tableware, lenses, sunglasses, furniture, mobile phones and thermal paper (EFSA, 2015; Ostberg et al., 2010; Tomza-Marciniak et al., 2018).

In recent years, numerous epidemiological studies have revealed associations between BPA and various civilization diseases, including cardiovascular disorders, obesity, diabetes, metabolic disorders, cancer, and infertility (Rochester, 2013). Due to its ability to mimic estrogen, BPA is recognized as an endocrine disruptor. This disruptive activity arises from the structural similarity of phenol groups present in both BPA and estradiol, enabling BPA to activate estrogenic pathways within the body (Rubin, 2011; Adegoke et al., 2020). Some studies consider the increased exposure of people to endocrine disruptors to be one of the reasons for the decrease in sperm concentration in the human population over the last 40 years (Cannarella et al., 2022).

The findings of these studies led to the ban on the use of BPA in the production of baby bottles in several countries (Canada, 2008; France, 2010; European Union, 2011). Furthermore, in certain countries, BPA's usage in food and beverage packaging has been completely prohibited (France from 2015). These regulatory actions reflect a response to the identified health concerns associated with BPA exposure.

After the implementation of these bans, manufacturers began to substitute BPA with structurally similar substances such as bisphenol S (BPS), bisphenol F (BPF) or bisphenol AF (BPAF). The use of these analogues is not regulated and considering their structural similarity with BPA they can potentially exhibit similar effects as BPA.

Bisphenol AF is a fluorinated derivate of BPA used in production of gaskets, hoses, polycarbonate copolymers and also high temperature composites. This substance was also detected in rivers (Song et al., 2012). Bisphenol F is even more commonly used than BPAF and is even contained as a natural product in mustard (Zoller et al., 2016). Several studies have shown that BPF and BPAF can cause endocrine disruption and exhibit reproduction toxicity or neurotoxicity (Chen et al., 2016; Liu et al., 2021). However, the most widespread of these analogues is BPS. Bisphenol S is used as a replacement for BPA in baby bottles (Simoneau et al., 2011) and thermal paper (U.S. Environmental Protection Agency, 2015) and all the three analogues are used in the production of plastics and epoxy resins (National Toxicology Program, 2015).

The majority of human biomonitoring studies have primarily concentrated on assessing bisphenols (BPs) exposure through urine measurements, which primarily reflects the excretion rate and biodegradation of BPs. However, seminal plasma stands out as the optimal choice for evaluating the pathophysiological effects of BPs on reproductive organs and, in particular, the testes (Vitku et al., 2016; Sanchez-Resino et al., 2023). In assessing the impact of pollutants on male reproduction, it is important to analyse chemicals directly from ejaculate (Wang et al., 2022; Sanchez-Resino et al., 2023). Only in a few studies, the bisphenols were detected directly in seminal plasma and a negative association between sperm count, concentration, morphology and seminal BPA was observed, but interestingly, the same associations were not observed with blood plasma BPA (Vitku et al., 2016).

In various species (including humans), a direct effect of bisphenols on sperm has been observed, manifested by the induction of increased oxidation and pro-apoptotic mitochondrial dysfunction, which led to impaired motility, viability, and a higher degree of sperm DNA fragmentation (Barbonetti et al., 2016). Additionally, in human sperm, it has been found that bisphenols (BPG, BPAF, BPC, BADGE, BDP, and BPBP) can affect the regulation of calcium channels known as Cation channel of Sperm (CatSper), which are specific for sperm (Rehfeld et al., 2020). CatSper are activated in the physiological environment by female progesterone produced in the cumulus cells and play a key role in acquiring the fertilizing ability of sperm (Lishko et al., 2011). Although BPA increases reactive oxygen species (ROS) production and thus oxidative stress in sperm, a decrease in ROS production was detected for BPS or BPF conformers. This suggests that these analogues may not initiate pro-oxidative and pro-apoptotic mechanisms in the same way as BPA (Nguyen et al., 2022).

The aim of this work was the detection and quantification of BPA and its three structural analogues (BPS, BPAF, BPF) in the seminal plasma of patients of the Centre of Reproductive Medicine of the University Hospital Brno, Czech Republic. Based on the analysis of the spermiogram and the DNA integrity of the patients' sperm, the relationship between the level of detected bisphenols and the parameters of the spermiogram was established. Moreover, the study explored the relationship between patients' occupations and the concentrations of bisphenols in their seminal plasma.

2. Material and methods

2.1. Study design

The study included a total of 358 male partners from couples who presented to Centre of Reproductive Medicine of University Hospital Brno, Czech Republic. Samples were collected between January 2019 and December 2021. A questionnaire was used to collect information including lifestyle factors, occupation, personal information and medical history. After study explanation, a total of 358 patients agreed with their participation in this study and provided signed informed consent for their participation. For spermiogram analyses, 9 patients were excluded. One patient had undergone vasectomy, another had an unsuccessful masturbation attempt (without providing a sample), six patients reported a use of antidepressants, other medications or disease with potential effects on spermiogram results and one patient left the study before providing a sample. Spermiogram analyses were made for 349 patients, anonymized list of all patients is available in Suppl4.

The study was approved by the Ethics Committee of University Hospital Brno (Approval No. 10–170221/EK) in accordance the Declaration of Helsinki (2000).

2.2. Semen collection

Semen samples were collected by manual masturbation after 2–8 days of sexual abstinence into a sterile wide-mouth container. All samples were incubated in room temperature for 60 min until total liquefaction. Before analyses of spermiogram, 0.5 mL of sample (if the sample was too small, a volume that contained at least 1 mil of spermatozoa) was stored in liquid nitrogen (−196 °C) for DNA analyses by using HALO sperm test. Seminal plasma sample was collected after spermiogram analyses. Immediately after spermiogram analyses, the samples were centrifuged, and seminal plasma was frozen in glass tube and stored at −20 °C until measurement. Small part of seminal plasma (200–400 μL) was prepared for ROS analyses (stored in −196 °C).

2.3. Semen analyses

Each patient underwent spermiogram analyses according to the World Health Organization laboratory manual (2010) (World Health Organization, 2010). Volume was assessed by weighing. After liquefaction of the ejaculate, a detailed spermiogram analysis was performed for each sample. In this study we focused on five semen quality parameters: ejaculate volume, sperm concentration, progressive motility, percent sperm with normal morphology and total sperm count (volume × sperm concentration). Details are described in Suppl1.

2.3.1. Sample preparation and bisphenol detection

Semen samples were collected in polypropylene cups and the collection material was shown to be bisphenols free by conducting leaching experiment using simulated semen fluid (Suppl1). To minimise sample contact with plastic materials, the seminal fluid samples were stored in glass vials at −20 °C before shipment to RECETOX trace

laboratory, where the analysis of bisphenols was performed.

The laboratory analysis involved solvent extraction using mixture of toluene and ethyl acetate (50/50; v/v) followed by separation with high-performance liquid chromatography, and detection by isotope-dilution tandem mass spectrometry (LC/MSMS). Further details of the analytical approach are described in Suppl1.

2.4. DNA integrity assessment

Sperm DNA fragmentation was assessed by sperm chromatin dispersion test (Halosperm G2 kit) according manufacturer's instruction. Strong staining is preferred to visualise the periphery of the dispersed DNA loop halos. Spermatozoa with big and medium halo were considered without fragmentation, while spermatozoa with small halo, without halo and degenerated were considered to be with fragmentation.

The DNA fragmentation indexes (DFI) were calculated by the form DFI (%) = *(fragmented spermatozoa + degenerated spermatozoa/total spermatozoa counted) × 100*. For the present study, a minimum of 600 spermatozoa per sample were scored under the 100 × objective of the microscope. To reduce the bias, two different technicians counted at least 300 spermatozoa each.

2.5. Determination of reactive oxygen species (ROS)

The level of hydrogen peroxide (one of the main ROS present in semen) was measured in semen samples using a commercially available kit. Total amount of H_2O_2 was determined using the Fluorometric Hydrogen Peroxide Assay Kit (Merck), according to manufacturer's manual. The fluorescent signal was read in a Fluostar Omega Microplate Reader (BMG Labtech, Ortenberg, Germany) at $\lambda_{ex} = 544$ nm and $\lambda_{em} =$ 590/10 nm.

2.6. Statistical evaluation

Since the study objective was to investigate the overall association between BPs levels in seminal plasma and the general trend of semen quality, we did not employ WHO cutoffs for classification to normal/abnormal semen quality. Instead, we focused on examining a continuous relation between seminal BPs levels and various parameters indicating semen quality. However we have excluded 36 of the 349 patients with potentially biased relation between the BPs and sperm quality. This was mainly due to fever in last month (23 patients), antidepressant treatment (6 patients), ongoing chemotherapy (2 patients), radiological exposure (2 patients), application of testosterone (1 patient) or seminal vesicles inflammation (1 patient). One patient was excluded due to his recent work with elemental sulfur for wine barrels sulfurization. Other 7 patients were removed due to a potential mismatch of the samples during the laboratory analysis, resulting in final 306 patients used for the statistical modeling. See Suppl4 for detailed reasons of the patients' exclusion. Generalized linear models (GLMs) were used to identify and quantify this relation while accounting for potential confounding factors. In case of DNA integrity, morphology and progressive motility, models with quasibinomial distribution and logit link function were used; in case of total amount of sperms, volume and concentration of sperm, models with quasipoisson distribution and log link function were used. Prior the model fitting, BPs concentrations were transformed using decadic logarithm to reach less skewed statistical distributions.

To handle an uncertainty originating from values under the limit of quantification (LOQ), left-censored values of very low bisphenol concentration were repeatedly imputed. The imputation was repeated 1000 times in loops deploying triangular statistical distribution of probability between zero and the LOQ. In each loop, a new GLM was fitted. Results were derived as medians of the 1000 GLM coefficient's estimates (β) and corresponding variable's p-values. Concepts of relative risk (RR, for logarithmic models) and odds ratio (OR, for logit models) were also used to express the values of β coefficients in a standardized way. BMI, smoking, ROS, age and sexual abstinence were used as confounders of the models (as discussed further).

For BPF and BPAF, this type of quantitative modeling is uncertain since more than 50% of their values are below the censoring limit. In such case, it is suitable to curb the statistical outcome on a qualitative comparison of dependent variable values between two groups of censored vs. uncensored values. Analysis of variance (ANOVA) was used to compare values of dependent variables between these groups, adjusted for the same set of confounders as in the modeling: BMI, smoking, ROS, age and ejaculation abstinence. The same qualitative analysis was made also for BPA and BPS and compared to the results of the quantitative modeling.

2.6.1. Confounders selection

Various confounding factors have the potential to impact the association between exposure to BPs and spermiogram parameters. These factors can influence both the levels of BPs and the quality of sperm. Incorporating these confounding variables into the assessment enhances the precision and reliability of the results, guarding against erroneous conclusions that may incorrectly attribute the observed relationship. For example it was observed that the adverse effect of bisphenol presence on sperm count was more prominent in obese men (Hu et al., 2017; Ghayda et al., 2019). The influence of patients' age on spermiogram results has been tested on multiple occasions, revealing a significant correlation. The peak in sperm parameters was observed between the ages of 30–35, with a notable reduction in these values observed after the age of 55 (Levitas et al., 2007).

Likewise, the duration of ejaculation abstinence was chosen as one of the confounding factors, as it has the potential to influence the ultimate spermiogram parameters (Hanson et al., 2018). The adverse impact of smoking on sperm parameters has been consistently demonstrated and based on meta-analyses, it can be concluded that cigarette smoking is associated with a decrease in sperm count, motility, and morphology (Sharma et al., 2016).

Reactive oxygen species (ROS) play a crucial role in sperm maturation, capacitation, hyperactivation, and fertilization. However, when ROS concentrations become supra-physiological, they can lead to lipid peroxidation, DNA fragmentation, and sperm apoptosis. Among ROS, hydrogen peroxide represents principal member and its elevated level is responsible for a poor sperm quality (Alahmar et al., 2019). Is it generally accepted that oxidative stress is an important cause of male factor infertility (Dutta et al., 2019).

For the reasons mentioned above we identified BMI, ROS, age, smoking and ejaculation abstinence as confounders during statistical evaluation. The statistical analysis of the confounders' impact on spermiogram parameters is presented in Suppl2 for each confounder individually.

3. Results

3.1. Characteristic of patients and semen quality parameters

With regards to patient age and spermiogram parameters, this subpopulation showed no significant distinctions when compared to the center's patient population examined during the same time period. The median sperm concentration, total sperm count, as well as progressive motility within this study's cohort closely resembled the parameters observed in all other patients (Table 1).

3.2. The content of BPs in the seminal plasma of men in the Czech Republic

Total number of 349 samples of seminal plasma were analysed with 4 bisphenols detected: BPA and its three common replacements BPS, BPF, BPAF with their concentration decreasing in that order. In 6 samples all

Table 1
Comparison of characteristics of participants included in the study to the whole patients' population analysed during 2019–2021.

Characteristics (median | IQR)	Included participants (n = 349)	Whole population (n = 2481)
Age (years)	32.0 | 9.0	32.0 | 10.0
Ejaculate volume (ml)	3.4 | 2.3	3.2 | 2.3
Total count of spermatozoa (mil)	122.2 | 152.5	105.0 | 149.0
Sperm concentration (mil/ml)	38.0 | 45.0	34.0 | 42.0
Progressive motility (%)	38.0 | 26.0	37.0 | 23.0
Normal morphology (%)	7.0 | 6.0	6.0 | 5.0
DNA integrity	19.0 | 12.8	NA

Integrity of DNA was not assessed within the whole patients' population.
Median BMI of the participants was 25.1 with IQR of 4.2. In the respondent group, 40.7% of men were overweight (BMI 25–30) and 11.2% of men were obese (BMI>30).

BPs were under their quantification limits, and in 20 samples all 4 BPs were quantified.

BPA was quantified in 267 samples (76.5%), similarly to BPS which was detected in 270 samples (77.4%). In case of BPF, it was only 50 samples (14.4%) and BPAF was detected in 80 (23.9%) (Fig. 1). While a median concentration of 0.061 ng/ml was measured for BPA, the median for BPS was lower (0.024 ng/ml), for BPF it was 0.00071 ng/ml and for BPAF even only 0.00049 ng/ml (Fig. 2).

Based on these results, BPA and BPS are fully eligible for the statistical assessment using GLMs, while GLM results of BPF and BPAF were only considered valid if confirmed by ANOVA applied on frequency of nondetects.

Respondents (n = 349) completed their occupation in the questionnaire and also stated whether they work in a dusty environment (n = 105), whether they are in daily contact with money and receipts (n = 28), work in an office (n = 194) or in a chemical factory (n = 8). All patients filled out a questionnaire regarding their occupation. According to their profession, the patients were classified into potentially risk groups (dusty environment, office, chemical production or workers with receipts and money), a combination of two or even three risk environments was detected in several patients (Suppl3).

During the statistical analysis, the relationship between the work environment and the concentration of the measured bisphenols was studied. No dependence was found between working in a dusty environment, working in an office environment, working with money and

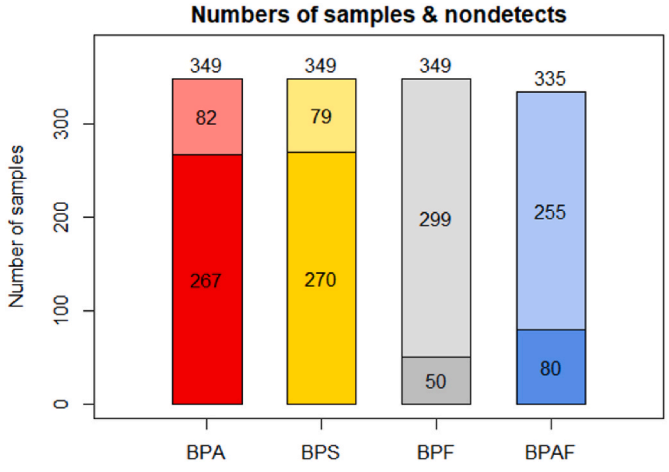

Fig. 1. Number of samples in which BPs were detected (dark shade of color) and nondetects (BPs below level of quantification – light shade of the color). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

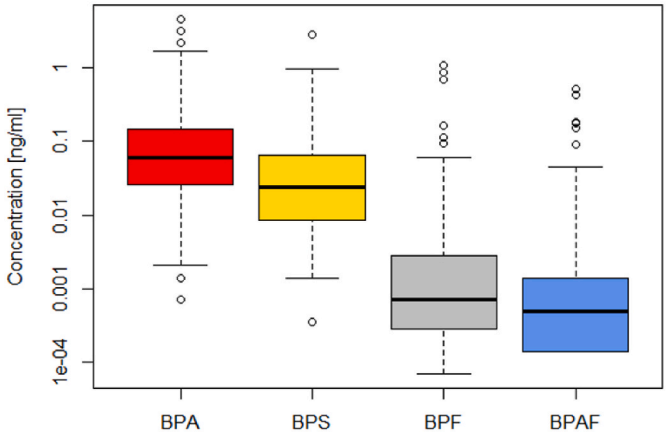

Fig. 2. Detected concentrations of BPs. Thick black lines show median values, boxes span between 25th and 75th percentile and whiskers expand 1.5 × IQR from box margins. Outliers are shown as dots out of the whiskers. Median values were 0.061 ng/ml for BPA, 0.024 ng/ml for BPS, 0.00071 ng/ml for BPF and 0.00049 ng/ml for BPAF.

receipts or working in chemical production in relation to the detected concentration of BPS, BPA, BPF or BPAF (Suppl3).

However, of the 5 patients with the highest BPA levels (all had the sum of all BPs greater than 3 ng/ml) there were 3 who worked in a dusty environment and 1 who worked with money and receipts daily, one reported working with both money and also in dusty environments. In the group of patients with the highest detected levels of all bisphenols (from 4.751 to 0.5 ng/ml), there were 28 patients divided as follows: 10 dust, 14 office, 2 money and receipts, 1 chemical production and 1 work with sulfur. It is interesting that the second highest (4.577 ng/ml) and the ninth highest (1.712 ng/ml) mentioned a similar profession, which otherwise does not appear at all in the cohort, namely green maintenance-gardener.

No bisphenol was detected in 6 respondents. In terms of professions, it was divided as follows: 3 office profession (50%), 1 × dusty environment, 1 × student and 1 × hospital assistant.

3.3. Spermiogram parameters

Results of all (24) generalized linear models including bisphenols as well as confounder's β coefficients, p-values of the GLMs, histogram of residuals, values of Spearman's correlation coefficients between the BPs and spermiogram parameters, ρ and corresponding p-values are available in Suppl3. The main results consisting of bisphenols β coefficients, RRs or ORs and corresponding p-values are listed in Table 2 and visualized in Fig. 3.

3.3.1. Sperm concentration, volume and total sperm count

The statistical analysis deploying GLMs on 95% level of confidence revealed no significant relation between concentration of sperm and the BPs (as presented in Table 2). However, it's noteworthy that all estimated β coefficients were negative, implying that elevated concentrations of BPs tend to have a general negative impact on sperm concentration.

In case of total sperm count and volume, we did not find any significant association between BPA concentration and these parameters using GLM, however the β coefficients were still negative and there were significant differences between men with BPA levels below and above LOQ (men with BPA above LOQ had in average 86% of sperm volume and 65% of sperm count compared with men with BPA below LOQ). For BPS level, there was a statistically significant negative dependency between the BPS concentration and corresponding ejaculate volume and the total count of sperm: increasing BPS concentration by one order of

Table 2
β-coefficients and p-values of Generalized Linear Models.

	BPA	BPS	BPF	BPAF
Ejaculate volume	β = −0.042 p = 0.271 RR = 0.96 (0.89–1.03)	**β = –0.094 p = 0.013*** **RR = 0.91 (0.85–0.98)**	β = 0.025 p = 0.471 RR = 1.03 (0.96–1.10)	β = 0.005 p = 0.846 RR = 1.02 (0.93–1.06)
Total sperm count	β = −0.125 p = 0.080 RR = 0.88 (0.77–1.01)	**β = –0.167 p = 0.018*** **RR = 0.85 (0.74–0.97)**	β = −0.020 p = 0.759 RR = 0.98 (0.86–1.12)	β = −0.010 p = 0.854 RR = 0.99 (0.88–1.12)
Sperm concentration	β = −0.098 p = 0.114 RR = 0.91 (0.80–1.02)	β = −0.095 p = 0.126 RR = 0.91 (0.81–1.03)	β = −0.014 p = 0.793 RR = 0.99 (0.88–1.10)	β = −0.019 p = 0.729 RR = 0.98 (0.88–1.09)
Sperm progressive motility	**β = –0.167 p = 0.005**** **OR = 0.85 (0.75–0.95)**	β = 0.044 p = 0.491 OR = 1.04 (0.92–1.18)	β = 0.024 p = 0.696 OR = 1.01 (0.91–1.16)	β = −0.044 p = 0.452 OR = 0.96 (0.86–1.07)
Sperm morphology	**β = –0.174 p = 0.001***** **OR = 0.84 (0.76–0.93)**	β = −0.036 p = 0.495 OR = 0.96 (0.87–1.07)	β = −0.030 p = 0.532 OR = 0.97 (0.88–1.07)	β = −0.030 p = 0.509 OR = 0.97 (0.89–1.06)
DNA Integrity	β = 0.094 p = 0.113 OR = 1.10 (0.98–1.23)	β = −0.081 p = 0.155 OR = 0.82 (0.82–1.03)	**β = –0.129 p = 0.014*** **OR = 0.88 (0.79–0.97)**	β = 0.015 p = 0.757 OR = 1.02 (0.92–1.12)

Results significant on 95% confidence level are marked with asterisks, depending on their p-values: p < 0.050 *; p < 0.010 **; p < 0.001 *** and highlighted with the green frames.

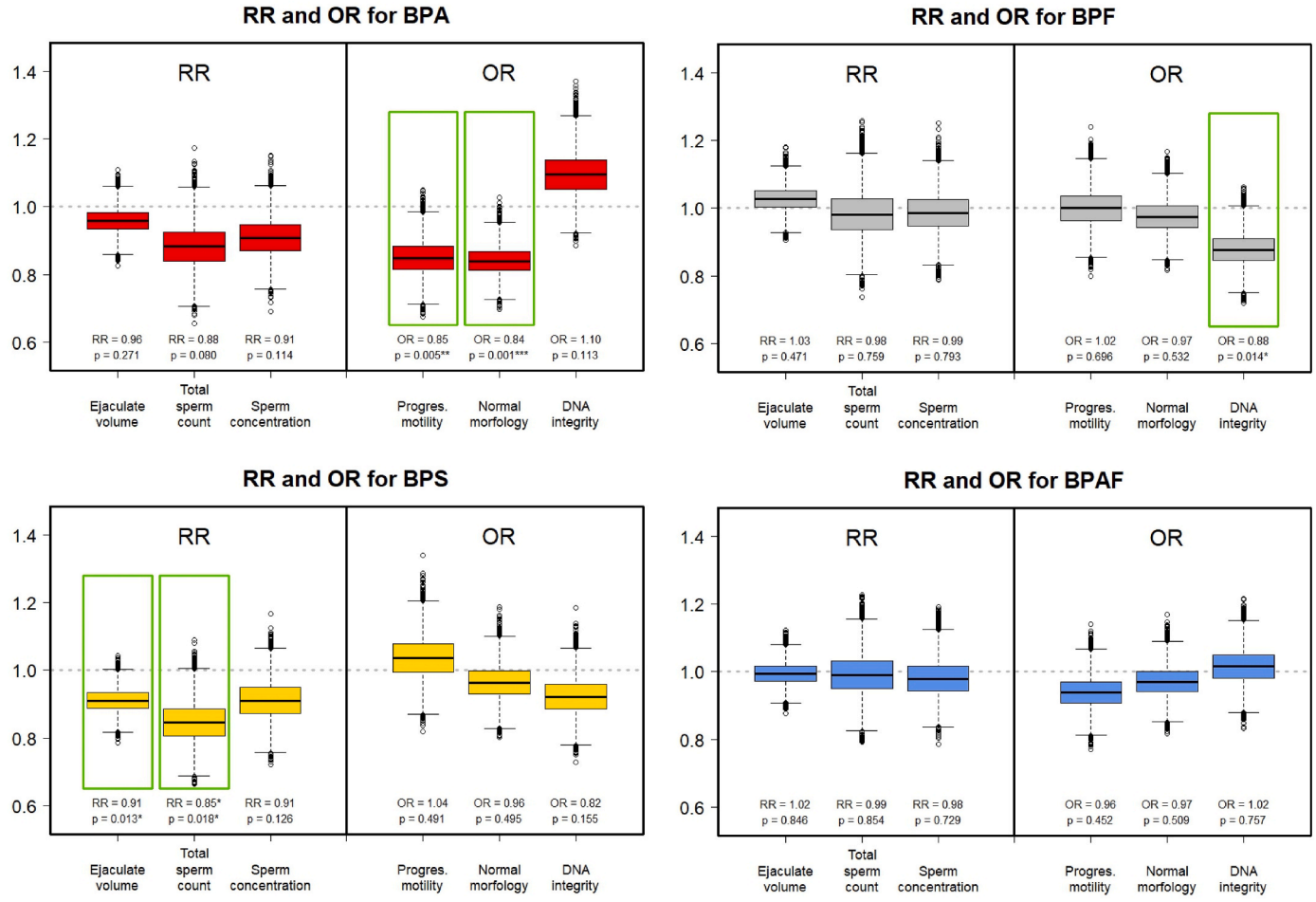

Fig. 3. Graphs of relative risks (RR) and odds ratios (OR) of sperm parameters based on generalized linear models. Thick black lines show median values, boxes span between 25th and 75th percentile and whiskers expand 1.5 × IQR from box margins. Outliers are shown as dots out of the whiskers. The grey horizontal dashed line highlights the value of 1: RR or OR on that line indicates no influence of the bisphenol on assessed sperm parameter. If the value of RR or OR is below that line, an increase of the bisphenol causes drop of the parameter, if the value of RR or OR is above that line, an increase of the bisphenol causes growth of the parameter. Results significant on 95% level of confidence are highlighted with green frames. Variability of the RR and OR values is caused by both the uncertainty within the GLMs and uncertainty of imputation of values below the limits of quantification. Primary data are presented in Suppl4. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

magnitude (ie. tenfold) leads to a mean relative decrease of sperm volume by 9% and sperm count by 5%. Considering the BPS concentration ranges more than 4 orders of magnitude, this leads to a decrease in sperm volume up to 50% and sperm count up to 20%. Analogous difference was observed between men with the BPS concentration below and above LOQ (men above LOQ in average had only 60% of sperm count of men below LOQ and also the sperm concentration was about 34% lower). No significant dependencies were observed between BPF and BPAF concentration, ejaculate volume, sperm count and concentration neither using GLM or ANOVA (Table 3).

Table 3
Differences Δ estimated by ANOVA between individuals with bisphenol concentration above and below LOQ (as decadic logarithms $\log_{10}$ (above)–$\log_{10}$ (below)).

	BPA	BPS	BPF	BPAF
Ejaculate volume	**Δ = –0.065 p = 0.014***	Δ = −0.037 p = 0.149	Δ = 0.022 p = 0.613	Δ = −0.018 p = 0.355
Total sperm count	**Δ = –0.186 p = 0.013***	**Δ = –0.218 p = 0.004****	Δ = −0.038 p = 0.526	Δ = −0.111 p = 0.112
Sperm concentration	Δ = −0.116 p = 0.089	**Δ = –0.176 p = 0.012****	Δ = −0.065 p = 0.359	Δ = −0.089 p = 0.185
Sperm progressivemotility	Δ = −0.044 p = 0.081	Δ = −0.010 p = 0.683	Δ = −0.013 p = 0.535	Δ = −0.034 p = 0.156
Sperm morphology	**Δ = –0.098 p = 0.016***	Δ = −0.049 p = 0.212	Δ = −0.013 p = 0.887	Δ = −0.018 p = 0.601
DNA Integrity	Δ = 0.049 p = 0.111	Δ = −0.008 p = 0.780	Δ = −0.068 p = 0.162	Δ = 0.013 p = 0.595

Results significant on 95% confidence level are marked with asterisks, depending on their p-values: $p < 0.050$ *; $p < 0.010$ **; $p < 0.001$ *** and highlighted with the green frames.

3.3.2. Sperm motility

Sperm motility is a very good and sensitive parameter that can be influenced by several factors, including the composition of the seminal plasma. For this reason, it is considered one of the important parameters accompanying the direct effect of substances in seminal plasma on sperm. Total and progressive motility were monitored and the dependence between the level of bisphenols and sperm motility was evaluated. BPA concentration was found to significantly ($p = 0.005$) negatively affect the percentage of progressively motile spermatozoa and total motility of spermatozoa in ejaculate. GLM for BPA provided odds ratio of 0.85 (95% confidence interval 0.76 to 0.96) which reliably shows a decrease of sperm motility of about 15% per increase of BPA concentration by one order of magnitude (ie. tenfold).

In case of BPA analogues (BPS, BPF and BPAF), no statistically significant correlations were found between the concentration of these substances and the progressive sperm motility neither using GLM or ANOVA.

3.3.3. Sperm morphology

Sperm morphology is a parameter that has a good informative value regarding the state of the sperm and the level of spermatogenesis in general. It is an important sign of good spermatogenesis of male sperm. Morphological evaluation of sperm resulted in relation to bisphenol concentrations similar to the concentration or motility. Statistically significant negative dependency was identified by GLM between the detected BPA level and the proportion of morphologically normal sperm. Odds ratio for BPA is 0.84, ie. an increase of the BPA concentration by one order of magnitude leads to roughly 16% decrease of morphologically normal sperm.

In the case of the BPA analogues (BPS, BPF, BPAF), no relationship was found between the detected level of these substances and the proportion of morphologically normal sperm neither any significant difference between men with BPA concentration below and above the LOQ.

3.3.4. DNA integrity

The integrity of DNA, which is not normally examined in a spermiogram, is a very important not only for the evaluation of the state of the sperm, but is also very important for the oocyte fertilization process and subsequent embryonal development. Nowadays, it is the DNA integrity disorders that are considered one of the most common causes of idiopathic infertility.

GLM identified a significant ($p = 0.014$) positive effect of increasing BPF concentration on the DNA integrity, with estimated OR of 0.88 (ie. increase of BPF concentration by one order of magnitude causes 12% decrease of fragmented sperm). Nevertheless, this result was not confirmed by ANOVA between values below and above LOQ (Table 3). Considering that 86% of BPF concentration values were below that limit, this effect should be considered as false-positive result affected by censored values imputation.

4. Discussion

In our study, we detected BPA and its three conformers within the general population of Czech men. Notably, at least one of these detected bisphenols was present in the seminal plasma of 98.3% of the subjects. This prevalence suggests a constant and continuous intake of these substances into the body, owing to their notably short half-life. This trend was observed consistently across various professions and age groups. Comparable findings were reported in the United States, where 92.6% of the population exhibited the presence of BPA or its conjugates in urine (Calafat et al., 2008). In Australia in 2012–2017, BPA was even detected in 100% of urine samples and BPS in 97% (Tang et al., 2020) underscoring the widespread exposure to these compounds.

The high detection frequency and abundance of BPA and BPS in seminal plasma observed in our study revealed that bisphenols exposure is widespread. BPA, BPS and BPF was detected in 95.7%, 89.4%, and 66.5% of randomly selected urine samples of U.S. adult. Median levels of BPA were higher (1.24 ng/ml) than BPF and BPS levels (0.35 and 0.37 ng/ml, respectively) (Lehmler et al., 2018). BPS, BPF, and BPAF have been detected in indoor dust and food and beverages in the USA and Asian countries (Liao et al., 2012b, Liao et al., 2013). In the indoor dust samples, BPA, BPS and BPF were the main compounds, accounting for >98% of total BPs. In urine samples from U.S. adults between 2000 and 2014, BPA was most frequently detected (74–99% of analysed samples; 0.36–2.07 ng/ml per year) followed by BPF (42–88%; 0.15–0.54 ng/ml) and BPS (19–74%, <0.1–0.25 ng/ml). BPAF was detected only rarely (<3% of all samples) (Ye et al., 2015).

Overall, the total BPA, BPS and BPF levels in seminal fluids were low when compared to the reported total bisphenol levels in urine in literature: BPA 1.49 ng/ml (Koch et al., 2012) BPA 1.24 ng/ml, BPS 0.37 ng/ml, BPF 0.39 ng/ml (Lehmler et al., 2018). When compared to other studies conducted on seminal plasma, we observed comparable results. Vitku et al. (2016) reported median BPA concentration of 0.085 ng/ml, by comparison, in our study we obtained 0.061 ng/ml. Only one previous study measured the presence of BPA, BPS and BPF in human seminal plasma (Louis et al., 2018). The study reported higher median concentration for BPA, BPS and BPF; 0.16, 0.110 and 0.038 ng/ml, respectively. Interestingly, this study detected BPF in 50% of samples ($n = 339$), we detected BPF only in 22% of samples ($n = 280$). This study also reported slightly different detection frequency for BPS (75%) compared to our study (88%). It is noteworthy that this study was conducted among the US male population, where different bisphenols exposure patterns are reported even for urine (Ye et al., 2015).

Study conducted in China among workers in chemical production showed a higher level of BPA in their urine and was associated with worse spermiogram parameters compared to men without exposure to BPA, mainly in the decrease in sperm concentration, total amount of sperm and motility (Li et al., 2011). In our cohort, we had only several study participants working in chemical factory ($n = 5$), however, no significant correlation with BPs was observed. In earlier studies, BPA was significantly higher in cashiers than that of non-cashiers, suggesting that thermal receipt paper is a potential source of BPA exposure in the workplace (Thayer et al., 2016). In 2015, the European Food Safety Authority (EFSA) reported that thermal paper is the second largest source of BPA exposure after the food ingestion (EFSA, 2015). Some suppliers have replaced BPA with its analogue BPS, which has been speculatively considered safer (Liao et al., 2012a). Within our cohort of patients whose professions involved handling money and receipts, no

significant difference in the levels of various bisphenols (BPs) was observed compared to the group that did not report this occupational exposure in the questionnaire (Suppl2).

Bisphenol A was associated with negative impact on male reproduction several times (Ješeta et al., 2021). In our study, we found negative impact of BPA on morphology and motility. Both of these parameters can be associated with direct impact of BPA on spermatozoa. In another study, it was reported that BPA had a negative impact on sperm motility and acrosomal reaction closely associated with downregulation and phosphorylation of fertility-related proteins in spermatozoa (Rahman et al., 2015). It was also presented that BPs induced mitochondrial disruption, apoptosis and DNA damage of Sertoli cells with impact on blood-testis barrier integrity (Adegoke et al., 2020). Bisphenols had also a direct effect on spermatozoa and increased oxidation and pro-apoptotic mitochondrial dysfunction of spermatozoa, which affected motility, viability, and sperm DNA fragmentation (Barbonetti et al., 2016). Also, an impact of BPs on sperm CatSper channel was documented (Rahveld et al., 2020). These mechanisms can be associated with lower motility and worse morphology in correlation with higher BPA level in seminal plasma.

Interestingly, we found a significant negative correlation between BPS concentration and total sperm count. It was shown that treatment with low doses of BPS reduced spermiogenesis and sperm count in male rats (Darghouthi et al., 2022). After chronic exposure to bisphenols, a reduction of testosterone concentration, structural changes of testicular tissue, reduced daily sperm production in rats were observed (Ullah et al., 2021). Men with detectable urinary levels of BPS exhibited a lower semen volume, reduced sperm concentration, and a lower total sperm count in contrast to men without detection of BPS (Ghayda et al., 2019). Chemical structure similar to estradiol giving BPA its estrogenic activities resulted in disruption of hypothalamus-pituitary-gonadal axis, lower LH secretion and hypostimulation of Leydig cell which led to lower local testosterone production and finally a negative impact on spermatogenesis (Barbonetti et al., 2016). This effect can be associated with the presented low volume and lower total amount of spermatozoa in correlation with BPS. BPs have also a direct negative impact on testes or spermatozoa. BPs can bind to estrogen receptors (ERμ, ERβ) and affect transcription and translation of genes and proteins, which has many effects on several types of cells (Adegoke et al., 2020). BPA can be an estrogen agonist or antagonist depending on type of cells (Wersinger et al., 1999). For example, in germ cells, apoptosis was induced by BPA via estrogen receptor and activation of IFNβ-XAF1-XIAP pathway (Jiang et al., 2018). BPs also bind to membrane G-protein coupled receptor (GPR30 – expressed on membrane of sperm cells) which starts phosphorylation of MAPK, PI3K, PKA triggering changes in cAMP, PKC and Ca levels resulting in cellular effects (Kitamura et al., 2005).

The effect of bisphenols on sperm DNA integrity has been studied for a long time with varying results. In 2022, it was discovered that BPA disrupts the histone-to-protamine transition in mice (Ryu et al., 2022). In clinical human studies, sperm DNA fragmentation was strongly correlated with aberrant sperm DNA protamination (Ni et al., 2016). In contrast to these findings, we did not observe any correlation between the concentration of BPs in seminal plasma and the integrity of spermatozoa DNA. In fact, we observed an opposing effect associated with the concentration of BPS in seminal plasma.

Although it seems that BPs can affect the state of the blood testis barrier (BTB) (Pena-Corona et al., 2021; Adegoke et al., 2020), the presence of BPs in seminal plasma is mediated primarily via the accessory glands, testes have only a minor role and the impact of BTB barrier on detected BPs concentration in seminal plasma is minimal (Ješeta et al., 2022). The study participants affirmed that their lifestyles and exposures were not different in the months before the samples were taken, suggesting relatively stable concentrations of BPA in the body. After all, measurement based on urine (or seminal plasma) detection at one point in time is a compromise that is accepted by many epidemiological studies (Lang et al., 2008).

5. Conclusion

In this study, we analysed 4 different bisphenols (BPA, BPS, BPA, BPAF) in the semen of Czech male population. Our findings revealed that both BPA and BPS were highly prevalent in the seminal plasma of Czech men, with a presence in over 90% of cases. Furthermore, we observed a negative correlation between the levels of BPA and BPS and spermiogram parameters. Specifically, elevated BPA levels were associated with decreased sperm motility and altered morphology. In the case of BPS, the study revealed a correlation with a reduction in ejaculate volume and a decline in the overall sperm count within the ejaculate.

The study presents, for the first time, a comprehensive analysis of BPA analogues directly in human semen, utilizing a substantial sample size. It also highlights the presence of BPS as a common replacement for BPA in the analysed samples. The fact that these compounds were detected in clinical samples suggests that newly appearing analogues of BPA can play important role in male reproduction health and should not be overlooked. This study not only enhances our understanding of the chemical composition of human semen but also provides insights into potential effects of BPs on spermatozoa.

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CRediT authorship contribution statement

Michal Jeseta: Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Jiri Kalina:** Writing – review & editing, Investigation, Data curation. **Katerina Franzova:** Methodology, Investigation, Data curation. **Sandra Fialkova:** Investigation. **Jan Hosek:** Investigation. **Lenka Mekinova:** Investigation. **Igor Crha:** Methodology. **Bartosz Kempisty:** Data curation. **Pavel Ventruba:** Methodology. **Jana Navratilova:** Writing – review & editing, Methodology, Investigation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Michal Jeseta reports financial support was provided by Brno University Hospital. If there are other authors, they 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.

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Appendix A. Supplementary data

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References

Adegoke, E.O., Rahman, M.S., Pang, M.G., 2020. Bisphenols threaten male reproductive health via testicular cells. Front. Endocrinol. 11, 624. https://doi.org/10.3389/fendo.2020.00624.

Alahmar, A.T., 2019. Role of oxidative stress in male infertility: an updated review. J. Hum. Reprod. Sci. 12, 4–18. https://doi.org/10.4103/jhrs.JHRS_150_18.

Barbonetti, A., Castellini, C., Di Giammarco, N., Santilli, G., Francavilla, S., Francavilla, F., 2016. In vitro exposure of human spermatozoa to bisphenol A induces pro-oxidative/apoptotic mitochondrial dysfunction. Reprod. Toxicol. 66, 61–67. https://doi.org/10.1016/j.reprotox.2016.09.014.

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.

Cannarella, R., Gül, M., Rambhatla, A., Agarwal, A., 2022. Temporal decline of sperm concentration: role of endocrine disruptors. Endocrine 79, 1–16. https://doi.org/10.1007/s12020-022-03136-2.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.L., Wu, Y., Widelka, M., 2016. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity-a review. Environ. Sci. Technol. 50, 5438–5453. https://doi.org/10.1021/acs.est.5b05387.

Darghouthi, M., Rezg, R., Boughmadi, O., Mornagui, B., 2022. Low-dose bisphenol S exposure induces hypospermatogenesis and mitochondrial dysfunction in rats: a possible implication of StAR protein. Reprod. Toxicol. 107, 104–111. https://doi.org/10.1016/j.reprotox.2021.11.007.

Dutta, S., Majzoub, A., Agarwal, A., 2019. Oxidative stress and sperm function: a systematic review on evaluation and management. Arab. J. Urol. 17, 87–97. https://doi.org/10.1080/2090598X.2019.1599624.

EFSA. Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Part I – exposure assessment. https://www.efsa.europa.eu/en/efsajournal/pub/3978 (accessed 21 January 2015).

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, 105050 https://doi.org/10.1016/j.envint.2019.105050.

Hanson, B.M., Aston, K.I., Jenkins, T.G., Carrell, D.T., Hotaling, J.M., 2018. The impact of ejaculatory abstinence on semen analysis parameters: a systematic review. J. Assist. Reprod. Genet. 35, 213–220. https://doi.org/10.1007/s10815-017-1086-0.

Hu, W., Dong, T., Wang, L., Guan, Q., Song, L., Chen, D., Zhou, Z., Chen, M., Xia, Y., Wang, X., 2017. Obesity aggravates toxic effect of BPA on spermatogenesis. Environ. Int. 105, 56–65. https://doi.org/10.1016/j.envint.2017.04.014.

Ješeta, M., Navrátilová, J., Franzová, K., Fialková, S., Kempisty, B., Ventruba, P., Žáková, J., Crha, I., 2021. Overview of the mechanisms of action of selected bisphenols and perfluoroalkyl chemicals on the male reproductive axes. Front. Genet. 12, 692897 https://doi.org/10.3389/fgene.2021.692897.

Ješeta, M., Franzová, K., Machynová, S., Kalina, J., Kohoutek, J., Mekiňová, L., Crha, I., Kempisty, B., Kašík, M., Žáková, J., Ventruba, P., Navrátilová, J., 2022. The bisphenols found in the ejaculate of men does not pass through the testes. Toxics 10, 311. https://doi.org/10.3390/toxics10060311.

Jiang, X., Yin, L., Zhang, N., Han, F., Liu, W.B., Zhang, X., Chen, H.Q., Cao, J., Liu, J.Y., 2018. Bisphenol A induced male germ cell apoptosis via IFNβ-XAF1-XIAP pathway in adult mice. Toxicol. Appl. Pharmacol. 355, 247–256. https://doi.org/10.1016/j.taap.2018.07.009.

Kitamura, S., Suzuki, T., Sanoh, S., Kohta, R., Jinno, N., Sugihara, K., Yoshihara, S., Fujimoto, N., Watanabe, H., Ohta, S., 2005. Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. Toxicol. Sci. 84, 249–259. https://doi.org/10.1093/toxsci/kfi074.

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.

Lang, I.A., Galloway, T.S., Scarlett, A., Henley, W.E., Depledge, M., Wallace, R.B., Melzer, D., 2008. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA 300, 1303–1310. https://doi.org/10.1001/jama.300.11.1303.

Lehmler, H.J., Liu, B., Gadogbe, M., Bao, W., 2018. Exposure to bisphenol A, bisphenol F, and bisphenol S in U.S. adults and children: the national health and nutrition examination survey 2013-2014. ACS Omega 3, 6523–6532. https://doi.org/10.1021/acsomega.8b00824.

Levitas, E., Lunenfeld, E., Weisz, N., Friger, M., Potashnik, G., 2007. Relationship between age and semen parameters in men with normal sperm concentration: analysis of 6022 semen samples. Andrologia US 39, 45–50. https://doi.org/10.1111/j.1439-0272.2007.00761.x.

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. https://doi.org/10.1016/j.fertnstert.2010.09.026.

Liao, C.Y., Kannan, K., 2013. Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. J. Agric. Food Chem. 61, 4655–4662. https://doi.org/10.1021/jf400445n.

Liao, C.Y., Liu, F., Kannan, K., 2012a. Bisphenol S., a new bisphenol analogue, in paper products and currency bills and its association with bisphenol A residues. Environ. Sci. Technol. 46, 6515–6522. https://doi.org/10.1021/es300876n.

Liao, C.Y., Liu, F., Guo, Y., Moon, H.B., Nakata, H., Wu, Q., Kannan, K., 2012b. Occurrence of eight bisphenol analogues in indoor dust from the United States and several asian countries: implications for human exposure. Environ. Sci. Technol. 46, 9138–9145. https://doi.org/10.1021/es302004w.

Lishko, P.V., Botchkina, I.L., Kirichok, Y., 2011. Progesterone activates the principal Ca2 + channel of human sperm. Nature 471, 387–391. https://doi.org/10.1038/nature09767.

Liu, J., Zhang, L., Lu, G., Jiang, R., Yan, Z., Li, Y., 2021. Occurrence, toxicity and ecological risk of bisphenol A analogues in aquatic environment - a review. Ecotoxicol. Environ. Saf. 208, 111481 https://doi.org/10.1016/j.ecoenv.2020.111481.

Louis, G.M.B., Smarr, M.M., Sun, L., Chen, Z., Honda, M., Wang, W., Karthikraj, R., Weck, J., Kannan, K., 2018. Endocrine disrupting chemicals in seminal plasma and couple fecundity. Environ. Res. 163, 64–70. https://doi.org/10.1016/j.envres.2018.01.028.

National Toxicology Program. NTP Brief on Bisphenol A [CAS NO. 80-05-07]. http://ntp.niehs.nih.gov/ntp/ohat/bisphenol/bisphenol.pdf (accessed 2 March 2015).

Nguyen, M., Sabry, R., Davis, O.S., Favetta, L.A., 2022. Effects of BPA, BPS, and BPF on oxidative stress and antioxidant enzyme expression in bovine oocytes and spermatozoa. Genes-Basel. 13, 142. https://doi.org/10.3390/genes13010142.

Ni, K., Spiess, A.N., Schuppe, H.C., Steger, K., 2016. The impact of sperm protamine deficiency and sperm DNA damage on human male fertility: a systematic review and meta-analysis. Andrology-US. 4, 789–799. https://doi.org/10.1111/andr.12216.

Peña-Corona, S.I., Vásquez Aguire, W.S., Vargas, D., Juárez, I., Mendoza-Rodríguez, C.A., 2021. Effects of bisphenols on Blood-Testis Barrier protein expression in vitro: a systematic review and meta-analysis. Reprod. Toxicol. 103, 139–148. https://doi.org/10.1016/j.reprotox.2021.06.008.

Peretz, J., Vrooman, L., Ricke, W.A., Hunt, P.A., Ehrlich, S., Hauser, R., Padmanabhan, V., Taylor, H.S., Swan, S.H., VandeVoort, C.A., Flaws, J.A., 2014. Bisphenol A and reproductive health: update of experimental and human evidence, 2007-2013. Environ. Health Perspect. 122, 775–786. https://doi.org/10.1289/ehp.1307728.

Rahman, M.S., Kwon, W.S., Lee, J.S., Yoon, S.J., Ryu, B.Y., Pang, M.G., 2015. Bisphenol-A affects male fertility via fertility-related proteins in spermatozoa. Sci. Rep. 5, 9169. https://doi.org/10.1038/srep09169.

Rehfeld, A., Andersson, A.M., Skakkebæk, N.E., 2020. Bisphenol A diglycidyl ether (BADGE) and bisphenol analogs, but not bisphenol A (BPA), activate the CatSper Ca2 + channel in human sperm. Front. Endocrinol. 11, 324. https://doi.org/10.3389/fendo.2020.00324.

Rochester, J.R., 2013. Bisphenol A and human health: a review of the literature. Reprod. Toxicol. 42, 132–155. https://doi.org/10.1016/j.reprotox.2013.08.008.

Rubin, B.S., 2011. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J. Steroid Biochem. Mol. Biol. 127, 27–34. https://doi.org/10.1016/j.jsbmb.2011.05.002.

Ryu, D.Y., Pang, W.K., Adegoke, E.O., Rahman, M.S., Park, Y.J., Pang, M.G., 2022. Abnormal histone replacement following BPA exposure affects spermatogenesis and fertility sequentially. Environ. Int. 170, 107617 https://doi.org/10.1016/j.envint.2022.107617.

Sánchez-Resino, E., Marquès, M., Gutiérrez-Martín, D., Restrepo-Montes, E., Martínez, M.Á., Salas-Huetos, A., Babio, N., Salas-Salvadó, J., Gil-Solsona, R., Gago-Ferrero, P., 2023. Exploring the occurrence of organic contaminants in human semen through an innovative LC-HRMS-based methodology suitable for target and nontarget analysis. Environ. Sci. Technol. 57, 19236–19252. https://doi.org/10.1021/acs.est.3c04347.

Sharma, R., Harlev, A., Agarwal, A., Esteves, S.C., 2016. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. Eur. Urol. 70, 635–645. https://doi.org/10.1016/j.eururo.2016.04.010.

Simoneau, C., Valzacchi, S., Morkunas, V., Van den Eede, L., 2011. Comparison of migration from polyethersulphone and polycarbonate baby bottles. Food Addit. Contam. 28, 1763–1768. https://doi.org/10.1080/19440049.2011.604644.

Song, S., Ruan, T., Wang, T., Liu, R., Jiang, G., 2012. Distribution and preliminary exposure assessment of bisphenol AF (BPAF) in various environmental matrices around a manufacturing plant in China. Environ. Sci. Technol. 46, 13136–13143. https://doi.org/10.1021/es303960k.

Tang, S., He, C., Thai, P.K., Heffernan, A., Vijayasarathy, S., Toms, L., Thompson, K., Hobson, P., Tscharke, B.J., O'Brien, J.W., Thomas, K.V., Mueller, J.F., 2020. Urinary concentrations of bisphenols in the australian population and their association with

the per capita mass loads in wastewater. Environ. Sci. Technol. 54, 10141–10148. https://doi/10.1021/acs.est.0c00921.

Thayer, K.A., Taylor, K.W., Garantziotis, S., Schurman, S.H., Kissling, G.E., Hunt, D., Herbert, B., Church, R., Jankowich, R., Churchwell, M.I., Scheri, R.C., Birnbaum, L. S., Bucher, J., 2016. Bisphenol A, bisphenol S, and 4-hydro xyphenyl 4-isopro oxyphenyl sulfone (bpsip) in urine and blood of cashiers. Environ. Health Perspect. 124, 437–444. https://doi.org/10.1289/ehp.1409427.

Tomza-Marciniak, A., Stepkowska, P., Kuba, J., Pilarczyk, B., 2018. Effect of bisphenol a on reproductive processes: a review of in vitro, in vivo and epidemiological studies. J. Appl. Toxicol. 38, 51–80. https://doi.org/10.1002/jat.3480.

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, 31. https://doi.org/10.1186/s12199-021-00954-0.

U.S. Environmental Protection Agency. Bisphenol A Alternatives In Thermal Paper. http://www2.epa.gov/saferchoice/partnership-evaluate-alternatives-bisphenol-thermal-paper (accessed 15 February 2015).

Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol a (BPA). Reprod. Toxicol. 24, 139–177. https://doi.org/10.1016/j.reprotox.2007.07.010.

Vandenberg, L.N., Maffini, M.V., Sonnenschein, C., Rubin, B.S., Soto, A.M., 2009. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocr. Rev. 30, 75–95. https://doi.org/10.1210/er.2008-0021.

Vitku, J., Heracek, J., Sosvorova, L., Hampl, R., Chlupacova, T., Hill, M., Sobotka, V., Bicikova, M., Starka, L., 2016. Associations of bisphenol A and polychlorinated biphenyls with spermatogenesis and steroidogenesis in two biological fluids from men attending an infertility clinic. Environ. Int. 89, 166–173. https://doi.org/10.1016/j.envint.2016.01.021.

Wang, A., Wan, Y., Zhou, L., Xia, W., Guo, Y., Mahai, G., Yang, Z., Xu, S., Zhang, R., 2022. Neonicotinoid insecticide metabolites in seminal plasma: associations with semen quality. Sci. Total Environ. 811, 151407 https://doi.org/10.1016/j.scitotenv.2021.151407.

Wersinger, S.R., Haisenleder, D.J., Lubahn, D.B., Rissman, E.F., 1999. Steroid feedback on gonadotropin release and pituitary gonadotropin subunit mRNA in mice lacking a functional estrogen receptor alpha. Endocrine 11, 137–143. https://doi.org/10.1385/ENDO:11:2:137.

World Health Organization, 2010. Semen analysis. In: WHO Laboratory Manual for the Examination and Processing of Human Semen. WHO Press, Geneva - Switzerland, pp. 7–114.

Ye, X., Wong, L.Y., Kramer, J., Zhou, X., Jia, T., Calafat, A.M., 2015. Urinary concentrations of bisphenol A and three other bisphenols in convenience samples of U.S. adults during 2000-2014. Environ. Sci. Technol. 49, 11834–11839. https://doi.org/10.1021/acs.est.5b02135.

Zoller, O., Brüschweiler, B.J., Magnin, R., Reinhard, H., Rhyn, P., Rupp, H., Zeltner, S., Felleisen, R., 2016. Natural occurrence of bisphenol F in mustard. Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk. Assess. A. 33, 137–146. https://doi.org/10.1080/19440049.2015.1110623.