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Association between chemical mixtures and female fertility in women undergoing assisted reproduction in Sweden and Estonia

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ABSTRACT

Objective: Women of reproductive age are exposed to ubiquitous chemicals such as phthalates, parabens, and per- and polyfluoroalkyl substances (PFAS), which have potential endocrine disrupting properties and might affect fertility. Our objective was to investigate associations between potential endocrine-disrupting chemicals (EDCs) and female fertility in two cohorts of women attending fertility clinics.

Methods: In a total population of 333 women in Sweden and Estonia, we studied the associations between chemicals and female fertility, evaluating ovarian sensitivity index (OSI) as an indicator of ovarian response, as well as clinical pregnancy and live birth from fresh and frozen embryo transfers. We measured 59 chemicals in follicular fluid samples and detected 3 phthalate metabolites, di-2-ethylhexyl phthalate (DEHP) metabolites, 1 paraben, and 6 PFAS in >90% of the women. Associations were evaluated using multivariable-adjusted linear or logistic regression, categorizing EDCs into quartiles of their distributions, as well as with Bayesian Kernel Machine Regression.

Results: We observed statistically significant lower OSI at higher concentrations of the sum of DEHP metabolites in the Swedish cohort (Q4 vs Q1, β = -0.21, 95% CI: −0.38, −0.05) and methylparaben in the Estonian cohort (Q3 vs Q1, β = -0.22, 95% CI: −0.44, −0.01). Signals of potential associations were also observed at higher concentrations of PFUnDA in both the combined population (Q2 vs. Q1, β = −0.16, 95% CI -0.31, −0.02) and the Estonian population (Q2 vs. Q1, β = −0.27, 95% CI -0.45, −0.08), and for PFOA in the Estonian population (Q4 vs. Q1, β = −0.31, 95% CI -0.61, −0.01). Associations of chemicals with clinical pregnancy and live birth presented wide confidence intervals.

Conclusions: Within a large chemical mixture, we observed significant inverse associations levels of DEHP metabolites and methylparaben, and possibly PFUnDA and PFOA, with OSI, suggesting that these chemicals may contribute to altered ovarian function and infertility in women.

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1. Introduction

The prevalence of reproductive health problems is increasing globally, with up to one in six women of reproductive age experiencing difficulties conceiving or carrying a pregnancy to term (Boivin et al., 2007; Mascarenhas et al., 2012). Despite the increasing use of assisted reproductive technologies (ARTs) among people with decreased fecundity, success rates of live birth have remained similar (Sunderam et al., 2019). Infertility is defined as an inability to conceive after 12 months of regular unprotected intercourse and it can be caused by female, male, mixed female/male factors, or unexplained mechanisms. Ovarian disorders account for infertility in about 1 in 4 infertile couples (Azziz et al., 2016; Luborsky et al., 2003). Considering the importance of hormones in ovarian function during development as well as in adult life, it is reasonable to assume that human-made chemicals that disrupt the endocrine system contribute to rates of infertility (Gore et al., 2015; Mínguez-Alarcón and Gaskins, 2017).

Endocrine disrupting chemicals (EDCs) are defined as "exogenous substances or mixtures that alter the functions of the endocrine system and consequently cause adverse effects in an intact organism, or its progeny, or subpopulations" (Kortenkamp et al., 2011; Zoeller et al., 2012). Although hundreds of chemicals have been flagged as suspected EDCs, only a few have been classified as such in the European Union (EU) (Demeneix and Slama, 2019). Particular attention has been given to known or suspected EDCs such as phthalates, bisphenols, and per- and polyfluoroalkyl substances (PFAS), which have been linked to clinical outcomes of fecundity and fertility (Mínguez-Alarcón and Gaskins, 2017; Hammarstrand et al., 2021; Rashtian et al., 2019). These chemicals are widespread and ubiquitously found in daily consumed products including personal care and household items, as well as in contaminated environments. Due to their potential to disrupt the endocrine system, they may adversely affect multiple health aspects, including reproductive health (Mínguez-Alarcón and Gaskins, 2017; Hammarstrand et al., 2021; Rashtian et al., 2019).

Our current knowledge of the potential effects of environmental chemicals on female fertility, however, is hampered by several factors. Firstly, most studies have investigated EDCs as they relate to clinical outcomes that involve both paternal and maternal factors, making it hard to disentangle the effects of potential EDCs on female fecundity. The identification of possibly modifiable factors that specifically relate to female infertility will represent an important step in providing recommendations to women seeking to improve fertility and informing chemical safety legislation. As such, it is important to evaluate how these chemicals relate to highly predictive biomarkers of female fertility such as the ovarian sensitivity index (OSI). OSI is a measurement of ovarian competence reflecting the response to the exogenous follicle-stimulating hormone (FSH) stimulation during ART, and it qualifies among a large number of variables as a major predictor of live birth (LB) (Huber et al., 2013; Vaegter et al., 2017, 2019). OSI correlates with established markers of ovarian reserve such as anti-Müllerian hormone (AMH), antral follicle count (AFC) and basal FSH levels, but it is more closely associated with LB rate probably because it reflects not only the remaining pool of oocytes but also a functional aspect of the ovaries (Weghofer et al., 2020; Revelli et al., 2020). Secondly, women are exposed to a mixture of several chemicals that act as a complex environmental exposure that can interact in the human body (Mínguez-Alarcón and Gaskins, 2017; Billionnet et al., 2012), but most studies have so far focused on the effects of individual chemicals, failing to capture this complexity. Switching the focus to mixture approaches accrues several advantages and has long been advocated from both a methodological as well as a biological standpoint (Dominici et al., 2010; Taylor et al., 2016; Kortenkamp, 2007; Drakvik et al., 2020). Thirdly, most of the current evidence on chemicals and fertility comes from single-centered studies, limiting the generalizability of research findings.

To address these issues, we conducted an epidemiological study including 333 women undergoing ART procedures at two fertility clinics in Sweden and Estonia. Associations were investigated between a mixture of known and suspected EDCs, including both non-persistent chemicals such as phthalates and parabens, and persistent chemicals such as PFAS, and indicators of fertility in assisted conception that include the OSI as a female-specific index, and established clinical outcomes such as live birth (LB) and clinical pregnancy (CP). This study was performed as part of the EU-funded project FREIA (van Duursen et al., 2020).

2. Methods

2.1. Study population

Embedded in two European cohorts in Sweden and Estonia, this study included a total of 333 women undergoing ART treatment. Participants from the Swedish cohort were recruited at the Carl von Linnékliniken in Uppsala from April to June 2016. Out of 244 eligible women (age: 21–43) 190 were recruited. Five declined while 185 accepted and were included in the study. The Swedish study was approved by the Swedish Ethical Review Authority (original license dnr 2015/798–31/2, amendments 2016/360–32 and 2016/1523–32). The Estonian cohort consisted of 148 women (age: 23–43) recruited at Nova Vita Clinic AS in Tallinn between February and November 2019. Out of 195 eligible women, 182 were recruited, and a final cohort of 148 women was selected based on the amount of follicular fluid expected to be required for all chemical measurements (>2 ml). The Estonian study was approved by the Research Ethics Committee of the University of Tartu (approval no 289/M-8). In both cohorts, women were provided with oral and written information about the study, and they signed an informed written consent form in accordance with the Declaration of Helsinki. In addition, women in the Swedish cohort filled in a short questionnaire regarding their lifestyle. Samples and data were pseudonymized with random codes and processed by relevant regulations (the Swedish data protection law PUL, the Swedish law on biobanking in healthcare, General Data Protection Regulation, and the Estonian data protection law).

2.2. Sample collection

In the Swedish cohort, follicular fluid containing all cellular material and without visible blood contamination was collected from the follicles. The first aliquot was always discarded to avoid possible dilution by the flushing medium left in the needle. The samples were pooled per patient, and centrifuged at 500g for 15 min. The Estonian cohort samples were also collected from the follicles and centrifuged at 300g for 10 min and then at 2000g for 10 min. The flushing medium was removed from the needle and the hose prior to the ovarian puncture to avoid any sample dilution. The cell-free follicular fluid samples were aliquoted, and delivered to the university on ice within 2 h and frozen at −80 °C. To control for possible contamination from the IVF laboratory environment, 33 blank samples were collected and subjected to chemical analysis. In the Swedish cohort, the blank samples consisted of G-Rinse (Vitrolife, Stocholm, Sweden) medium that was used to flush the needle (Wallace Single Lumen Oocyte Recovery System 17G, CooperSurgical Fertility and Genomics, Målov, Denmark) prior to ovum pick-up, and such blank samples were collected at multiple time points during the sample collection period. The blank sample for the Estonian cohort was an unused flushing medium with 10 IU/ml heparin (ORIGIO, CooperSurgical Fertility and Genomics) that was passed through an unused single lumen ovum aspiration needle and hose (Cook Medical LLC, Bloomington, IN, USA).

2.3. Exposure assessment

Quantitative analyses of all chemicals were conducted in

laboratories using two methodologies for i) bisphenols, parabens, and phthalate metabolites and ii) PFAS. Tables S1 and S2 in the Supplementary Material present a complete list of all assessed chemicals including the isotopically labelled internal standards. For both methods, isotope dilution liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used. Additional details can be found in the Supplementary Material.

To quantify phthalates and parabens, a deconjugation step was carried out before solid-phase extraction (SPE). The conjugated metabolites in the follicular fluid samples (200 μl) were hydrolyzed by β–glucuronidase for 180 min at 37 °C. After concentration and washing, the target compounds were eluted from 10 mg Oasis MAX cartridges with 1 ml 2% formic acid in methanol. The extracts were measured on an LC (ExionLC, Sciex) coupled with a Turbo V Ion source (ESI) operating in the negative ion mode prior to triple quadrupole mass selective detection (6500+, Sciex). The compounds were separated on a Phenyl-hexyl column (Kinetex, 100 × 2.1 mm, 1.7 μm, Phenomenex) by applying a gradient of 0.2 mM NH4F and 0.2 mM NH4F in acetonitrile.

To quantify PFAS, aliquots of 200 μl follicular fluid were extracted with SPE using 10 mg Oasis WAX cartridges (Waters). The obtained extracts were analyzed on the same LC-MS/MS system, using an XBridge BEH C18 XP Column (2.5 μm, 2.1 × 150 mm) with 2 mM NH4CHOO and methanol – acetonitrile as gradient solvents. PFAS data from the Swedish cohort were previously quantified in a different laboratory (Björvang et al., 2022), and we, therefore, re-analyzed 10 samples with the current methods to ensure comparability, detecting negligible differences.

Our exposure assessment covered 59 chemicals (10 bisphenols, 6 parabens, 16 phthalate metabolites, and 27 PFAS) in the Estonian cohort and 40 (10 bisphenols, 6 parabens, 16 phthalate metabolites, and 8 PFAS) in the Swedish follicular fluid samples. Because of the very high correlation levels between the four metabolites [mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP)] of di-2-ethylhexyl phthalate (DEHP) (Fig. S1) and same parental compound, their sum (ΣDEHP) was created by dividing each metabolite concentration by its molecular weight and then summing for statistical analysis. Geometric mean values were used for women who had two plasticizers assessments (n = 16) or two PFAS assessments (n = 15). After inspecting the distribution of all exposures, 11 chemicals with less than 10% of samples below the limit of quantification (LOQ) were evaluated in primary analyses. These included 4 phthalates: ΣDEHP, MEP, cxMiNP (the only secondary metabolite of DiNP with high detection levels; primary metabolites were not quantified), MOHiBP (a secondary metabolite of MiBP; primary metabolites were not quantified); methylparaben; and 6 PFAS: PFHxS, PFOA, PFOS, PFNA, PFUnDA, and PFDA. In addition, cxMiNCH was detected in high proportions only among Swedish women, and propylparaben only among Estonian women. Therefore, these two chemicals were only examined in stratified analyses.

2.4. Outcomes assessment

For both cohorts, data on reproductive health at baseline and treatment outcomes were retrieved from electronic health records. To assess female fertility, we used OSI as a continuous measure of ovarian response to stimulation (Huber et al., 2013), as well as the fertility treatment endpoints of CP and LB from fresh and cumulative (i.e., fresh and frozen) embryo transfers, evaluated as binary outcomes (yes/no). In both cohorts, AMH and FSH were assessed from blood taken during the infertility investigation, before any fertility treatment, while the follicular fluid was taken during ovum pick-up. The follow-up times for Swedish and Estonian cohorts, used to examine CP and LB rates, were 5 years and 2.5 years, respectively. The two cohorts followed similar procedures for outcome measurements assessment, albeit the hormonal stimulation protocol differed between the centers and is described below.

2.4.1. Ovarian stimulation and ovarian sensitivity index

In the Swedish cohort, basal AFC (bAFC), which is the total number of follicles with a size of 2–10 mm, was determined via ultrasound before stimulation. In addition, the concentration of AMH in serum was measured. Participants underwent either gonadotropin-releasing hormone (GnRH) agonist protocol (82%) using Suprecur (Suprecur, Cheplapharm Arzneimittel GmbH, Greifswald, Germany) or Synarela (Synarela, Pfizer, New York City, New York, USA) where the pituitary was desensitized starting the luteal phase, or GnRH antagonist protocol (18%) where GnRH antagonist Orgalutran (Orgalutran,N.V. Organon, Oss, The Netherlands) was given on Day 6 of menses. To stimulate follicle growth and oocytes maturation, recombinant FSH (rFSH, Gonal-F or Fostimon, Bemfola, Gedeon Richter Plc., Budapest, Hungary) and/or human menopausal gonadotropin (Menopur, Ferring Pharmaceuticals Ltd,Saint-Prex, Switzerland) were given from day 3 of menses. Once there were at least three follicles of ≥17 mm, human chorionic gonadotropin (hCG) was given. After 36–37 h, oocytes were retrieved through the transvaginal ultrasound-guided ovarian puncture.

In the Estonian cohort, ovarian hormonal stimulation was conducted according to the GnRH antagonist (Cetrotide, Merck, Darmstadt, Germany) protocol with the administration of rFSH (Gonal-F®, Merck; Bemfola, Gedeon Richter Plc). AFC was measured after stimulation (sAFC) 2–3 days before ovum pick-up, and AMH was only measured among participants demonstrating potentially diminished ovarian reserve. All patients underwent oocyte retrieval 36 h after hCG administration (Ovitrelle®, Merck) if at least two follicles were observed with a diameter of ≥18 mm.

OSI was calculated by taking the natural logarithm (*ln*) of the previously described formula to improve the normal distribution of the outcome (Huber et al., 2013):

$$OSI = ln((\text{number of oocytes retrieved})/(\text{total rFSH dose (IU)}) \times 1000)$$

2.4.2. Clinical pregnancy and live birth

One to two embryos were transferred into the uterus per cycle. The remaining embryos were frozen and thereafter preserved in liquid nitrogen. CP was defined by confirming the presence of a gestational sac and fetal heartbeat by ultrasound 4 weeks after positive human chorionic gonadotropin detection from blood. LB was defined as the birth of a live baby after at least 24 weeks of gestation. Both measures were evaluated as binary indicators of success from only fresh transfers as well as both fresh and frozen transfers.

2.5. Other variables

Using a direct acyclic graph (DAG) (Tennant et al., 2021), presented in Fig. S2, we identified a set of potential confounders to be evaluated in primary analyses, and other covariates that might lie on the exposure-outcome pathway (i.e., potentially mediators) that we evaluated in secondary analyses. Potential confounders available for participants from both cohorts included age, body mass index (BMI), parity, previous in-vitro fertilization (IVF) cycles (IVF/intracytoplasmic sperm injection), and their outcome, and infertility causes. These variables were retained in the primary models if their inclusion changed the exposure's coefficients by at least 10%. Women participating in the Swedish cohort also completed a self-administrated questionnaire with additional lifestyle questions, thus allowing us to evaluate whether additional potential confounders, as well as sources of exposure, were independently associated with fertility outcomes in this subpopulation. Specifically, we assessed smoking, fish intake, personal care product (PCP) use, infertility duration, alcohol consumption, and menstrual cycle regularity (assessed from patient records), using the same

change-in-estimate criterion. PCP use was assessed using the average score of the 4-point Likert scale assessing the frequency of use of 6 products. Other variables related to female fertility, such as AMH concentration, basal AFC, endometrial thickness, and thyroid-stimulating hormones (only available in the Swedish cohort), possibly lie on the exposure-outcome causal pathway (Fig. S2) and were therefore evaluated as potential mediators in secondary analyses.

2.6. Statistical analysis

Descriptive statistics were presented as mean (standard deviation, SD) or number (percentage) in the combined population and per cohort. We also conducted a correlation analysis on the selected chemicals by calculating Spearman correlation coefficients.

To examine the associations between potential EDCs and female fertility outcomes, we first used linear regression (for OSI, which was normally distributed) and logistic regression (for CP and LB), to independently evaluate each chemical in separate models adjusted for potential confounders. All models were evaluated in the combined population and stratified by cohort. Covariates that met the inclusion criterion were age, BMI, parity, and previous IVF. For the Swedish cohort we further adjusted for infertility duration, smoking, fatty fish intake, and PCP use. Smoking was not adjusted for in the analysis of the Estonian cohort due to the very small number of smokers. When analyzing the combined population, we used two approaches to account for structural differences between the Swedish and Estonian cohorts: 1) controlling for the cohort as an additional covariate in regression, and 2) using a linear mixed model with cohort as a random intercept. Analyses using these two approaches yielded consistent results and only the results from the first approach are therefore presented. To relax assumptions of linearity in dose-response associations, chemicals were evaluated as categorical exposures by calculating quartiles in the combined population. By only selecting chemicals with minimal levels of non-quantification, we did not use any imputation technique and evaluated all models using complete-cases analysis. To test the robustness of the findings, we also considered the possibility that MEHP might not be a biologically formed metabolite in the follicle but rather derived from unspecific hydrolysis during sample collection and processing, and we, therefore, conducted a sensitivity analysis excluding MEHP from ΣDEHP. In another sensitivity analysis, we excluded cases of infertility due to male causes as these might not be associated with exposure levels of EDCs in follicular fluid in this study. Finally, we conducted a secondary exploratory analysis further adjusting for covariates that might also act as mediators.

Next, to account for co-exposure confounding within chemicals and to address potential mixture effects, we jointly evaluated all chemicals as a chemical mixture. We first mutually adjusted for multiple chemicals in one single regression model. This approach, however, can be subject to substantial bias in the presence of high correlation (i.e., multicollinearity), which can be quantified by variance inflation factors (VIFs). To address this issue, we applied Bayesian Kernel Machine Regression (BKMR), a statistical approach specifically designed to evaluate complex mixtures of correlated chemicals. BKMR is a supervised non-parametric method that incorporates a variable selection approach within the estimation of individual dose-response associations as well as the overall effect of the chemical mixture and flexibly accounts for potential non-linear relationships and interaction effects (Bobb et al., 2015, 2018). We used the hierarchical version of BKMR (Bobb et al., 2015), which allows for informing the model of clusters of chemicals (i.e., phthalates and parabens versus PFAS). BKMR also allows for estimating an overall mixture effect that can be interpreted as the change in the outcome while jointly increasing each chemical by percentiles.

All analyses were performed with the statistical software R version 4.1 (R Foundation for Statistical Computing, Vienna, Austria). All tests were two-tailed, and p-values <0.05 were conventionally used to indicate statistically significant associations.

3. Results

3.1. Population characteristics

Table 1 reports descriptive statistics of covariates, in the combined population and stratified by cohort. There were some differences in the distribution of several variables between cohorts. Estonian were more frequently infertile due to female causes than the women in the Swedish cohort. In addition, lower endometrial thickness and AMH were observed in the Estonian cohort, even though AMH data were only available in 35 women with an indication of low ovarian reserve in the Estonian cohort. In total, we observed 106 CP from fresh, 155 CP from fresh/frozen, 93 LB from fresh, and 135 LB from fresh/frozen transfers. The Swedish and Estonian cohorts had similar OSI (mean values of 0.62 and 0.63, respectively) as well as probabilities of CP (36% and 38%, respectively) and LB (32% and 34%, respectively).

Out of the measured chemicals [10 bisphenols, 6 parabens, 16 phthalate metabolites, and 27 PFAS (8 PFAS in Swedish cohort)], 11 (3 phthalate metabolites, the DEHP metabolites, evaluated as a molar sum, 1 paraben, and 6 PFAS) were quantified in >90% of women and used for statistical analyses. Distributions of these chemicals are presented in Table 2. The LOQs and distributions for all chemicals analyzed can be found in Table S3. PFAS concentrations were generally higher in the Swedish cohort, and the range of reported values was much wider than in the Estonian samples. No substantially different patterns in phthalates and parabens distributions were observed between the Estonian and Swedish cohorts. Fig. 1 presents the correlation matrix of the 11 evaluated chemicals. We observed a strong correlation structure ($r > 0.5$) between the six PFAS chemicals, whereas phthalates and parabens were largely uncorrelated. Analysis of the blank samples suggests minimal contamination from the embryo laboratory environment.

3.2. Phthalates, parabens, and fertility outcomes

The associations between phthalates, parabens, and OSI are presented in Table 3. In the combined population, we observed lower OSI at higher chemical concentrations, with generally broad confidence intervals. Within the 2 individual cohorts, significant differences were observed for ΣDEHP in the Swedish cohort (Q4 vs Q1, $\beta = -0.21$, 95% CI: -0.38, -0.05) and methylparaben in the Estonian cohort (Q3 vs Q1, $\beta = -0.22$, 95% CI: -0.44, -0.01). Evaluating chemicals as a mixture by mutually adjusting for phthalates and parabens in the same model did not affect the results (Table S4). In addition, consistent results were obtained from the analyses further adjusting for potential mediators (Table S5), in the sensitivity analysis excluding MEHP from ΣDEHP (data not shown), and excluding cases of infertility due to male causes (Table S6).

Fig. 2 presents associations between phthalates and parabens, evaluated independently, and clinical outcomes. Higher cxMiNP concentration was associated with lower odds of CP (Q4 vs. Q1, OR = 0.48, 95% CI 0.23, 0.94) in the combined population. No other significant associations between phthalates and parabens and clinical outcomes were observed.

3.3. PFAS and fertility outcomes

Table 4 presents the associations of PFAS with OSI, where all chemicals were mutually adjusted for in one regression model because of their high correlation. Higher concentrations of PFAS were generally related to lower OSI, with statistically significant associations for PFUnDA in both the combined population (Q2 vs. Q1, $\beta = -0.16$, 95% CI -0.31, -0.02) and the Estonian population (Q2 vs. Q1, $\beta = -0.27$, 95% CI -0.45, -0.08), and for PFOA in the Estonian population (Q4 vs. Q1, $\beta = -0.31$, 95% CI -0.61, -0.01). Analysis using individual regression models for each chemical showed consistent results for PFUnDA (Table S7). Further adjusting for potential mediators (Table S8) and

Table 1
Characteristics of the study population, overall and by cohort.

	Combined population (n = 333)	Sweden (n = 185)	Estonia (n = 148)
Variables available in both cohorts			
Age, mean (SD)	34.8 (4.5)	34.4 (4.7)	35.2 (4.2)
BMI, mean (SD)	23.4 (3.5)	23.5 (3.5)	23.2 (3.6)
Smoking, n (%)			
Never	265 (79.6)	162 (87.6)	103 (69.6)
Former/Current	30 (9.0)	23 (12.4)	7 (4.7)
Missing	38 (11.4)	0	38 (25.7)
ICSI or conventional IVF, n (%)			
ICSI	180 (54.1)	94 (50.8)	86 (58.1)
Conventional IVF	153 (45.9)	91 (49.2)	62 (41.9)
Parity, n (%)			
0	202 (60.7)	106 (57.3)	96 (64.9)
≥1	131 (39.3)	79 (42.7)	52 (35.1)
Infertility cause, n (%)			
Both male and female	35 (10.5)	14 (7.6)	21 (14.2)
Female	132 (39.6)	54 (29.2)	78 (52.7)
Male	75 (22.5)	44 (23.8)	31 (20.9)
Unexplained	91 (27.3)	73 (39.5)	18 (12.2)
Previous IVF, n (%)			
No	192 (57.7)	91 (49.2)	101 (68.2)
Yes	141 (42.3)	94 (50.8)	47 (31.8)
Previous IVF children, n (%)			
No	281 (84.4)	153 (82.7)	128 (86.5)
Yes	52 (15.6)	32 (17.3)	20 (13.5)
Regularity of menses, n (%)			
Regular	260 (78.1)	160 (86.5)	100 (67.6)
Irregular	36 (10.8)	25 (13.5)	11 (7.4)
Missing	37 (11.1)	0	37 (25.0)
Other variables			
Infertility duration, mean (SD)	\	2.3 (1.6)	
Personal Care Products score[a], mean (SD)		2.9 (0.5)	\
Alcohol, n (%)			
Daily/weekly	\	43 (23.2)	\
Monthly		63 (34.1)	\
Seldom/never	\	76 (41.1)	\
Missing	\	3 (1.6)	\
Fatty fish, n (%)			
Daily/weekly		89 (48.1)	\
Monthly		71 (38.4)	\
Seldom/never	\	19 (10.3)	\
Missing	\	6 (3.2)	\
Anti-mullerian hormone (AMH) concentration,[b] mean (SD)	3.0 (2.9)	3.3 (3.0)	1.7 (1.7)
Thyroid stimulating hormones (TSH) concentration, mean (SD)	\	1.6 (0.8)	
Basal antral follicle count (bAFC),[c] mean (SD)	\	19.4 (11.9)	\
Stimulated antral follicle count (sAFC), mean (SD)		\	12.4 (9.6)
Endometrial thickness,[d] mean (SD)	11.1 (2.3)	11.6 (2.4)	10.5 (2.0)

[a] Calculated as a score of several personal care products usage.
[b] AMH in the Estonian cohort was only calculated for n = 35 (23.6%) women who demonstrated problems with ovarian reserve.
[c] bAFC had n = 21 (11.4%) missing values in the Swedish cohort.
[d] Endometrial thickness had n = 20 (13.5%) missing values in the Estonian cohort.

excluding cases of infertility due to male causes (Table S6) did not substantially affect the results (Table S8).

Fig. 3 presents associations between PFAS, mutually adjusted in one logistic regression model, and clinical outcomes. PFHxS was associated with lower odds of LB from fresh transfer (Q2 vs. Q1, OR = 0.35, 95% CI 0.12, 0.98; Q3 vs. Q1, OR = 0.31, 95% CI 0.10, 0.94) and lower odds of LB (Q2 vs. Q1, OR = 0.23, 95% CI 0.09, 0.76) from fresh/frozen transfers. In addition, a higher concentration of PFOA was related to lower odds of CP from the fresh/frozen transfers (Q4 vs. Q1: OR = 0.31, 95% CI 0.10, 0.92). Interestingly, we also noted a positive relation between PFUnDA concentration and LB from the fresh transfer (Q2 vs. Q1: OR = 3.18, 95% CI 1.11, 9.97). In both linear and logistic regression models mutually adjusting for PFAS, VIFs (range: 3.0 to 10.0 for the overall population) suggested that these results are likely affected by multicollinearity, thus mixture modeling, presented in the next subsection, was required to validate the results.

3.4. Bayesian Kernel Machine Regression (BKMR) analysis

Hierarchical BKMR was performed in a subsample of the combined population where complete data on all exposures (i.e., phthalates/parabens and PFAS) and confounders were available (n = 283). Since BKMR requires evaluating chemicals as continuous covariates, concentrations of chemicals were log-transformed prior to analysis. In addition, we fitted a BKMR model without the hierarchy (all exposures) and containing an option to allow for within-cohort differences to account for structural population differences. Negligible differences in the results were observed, and we, therefore, presented only results from hierarchical BKMR without random effects for cohort.

Dose-response associations between selected chemicals (i.e., those showing some signal in regression and mixture modeling) and OSI are presented in Fig. 4, showing overall inverse associations. However, all estimates presented broad credible intervals that included the null associations. The overall mixture effect is presented in Fig. 5, suggesting an inverse association between OSI and the chemical mixture. BKMR

Table 2
Distributions and levels of quantification of chemicals evaluated in primary analyses (ng/mL).

	Sweden (n = 185)	Estonia (n = 148)	Combined population (n = 333)
MEHP			
Mean (SD)	1.04 (1.65)	0.98 (0.40)	1.02 (1.28)
Median [Min, Max]	0.78 [0.48, 21.00]	0.9 [0.68, 3.60]	0.85 [0.48, 21.00]
Non-quantified, n (%)	25 (13.5%)	33 (22.3%)	58 (17.4%)
MECPP			
Mean (SD)	0.39 (1.85)	0.30 (0.21)	0.35 (1.38)
Median [Min, Max]	0.19 [0.06, 25.00]	0.23 [0.09, 1.60]	0.21 [0.06, 25.00]
Non-quantified, n (%)	3 (1.6%)	3 (2.0%)	6 (1.8%)
MEHHP			
Mean (SD)	0.08 (0.35)	0.06 (0.02)	0.07 (0.26)
Median [Min, Max]	0.04 [0.02, 4.70]	0.06 [0.04, 0.13]	0.05 [0.02, 4.70]
Non-quantified, n (%)	5 (2.7%)	8 (5.4%)	13 (3.9%)
MEOHP			
Mean (SD)	0.05 (0.25)	0.05 (0.02)	0.05 (0.21)
Median [Min, Max]	0.02 [0.01, 3.30]	0.05 [0.04, 0.17]	0.03 [0.01, 3.30]
Non-quantified, n (%)	10 (5.4%)	90 (60.8%)	100 (30.0%)
ΣDEHP[a]			
Mean (SD)	0.004 (0.002)	0.004 (0.002)	0.004 (0.002)
Median [Min, Max]	0.004 [0.0003, 0.014]	0.004 [0.0005, 0.014]	0.004 [0.0003, 0.014]
Non-quantified, n (%)	4 (2.2%)	1 (0.7%)	5 (1.5%)
MEP			
Mean (SD)	0.98 (1.10)	1.02 (1.54)	1.00 (1.31)
Median [Min, Max]	0.74 [0.29, 10.00]	0.6 [0.28, 12.00]	0.71 [0.28, 12.00]
Non-quantified, n (%)	4 (2.2%)	8 (5.7%)	12 (3.6%)
cxMiNP			
Mean (SD)	2.52 (7.03)	2.99 (6.62)	2.72 (6.85)
Median [Min, Max]	0.61 [0.10, 70.00]	0.55 [0.16, 37.00]	0.58 [0.10, 70.00]
Non-quantified, n (%)	3 (1.6%)	5 (3.4%)	8 (2.4%)
MOHiBP			
Mean (SD)	0.07 (0.05)	0.04 (0.02)	0.06 (0.04)
Median [Min, Max]	0.05 [0.02, 0.41]	0.04 [0.02, 0.16]	0.05 [0.02, 0.41]
Non-quantified, n (%)	13 (7.0%)	3 (2.0%)	16 (4.8%)
Methylparaben			
Mean (SD)	152.69 (653.76)	58.14 (39.08)	110.19 (487.43)
Median [Min, Max]	7.9 [0.13, 6000.00]	54 [0.16, 230.00]	30 [0.13, 6000.00]
Non-quantified, n (%)	5 (2.7%)	1 (0.7%)	6 (1.8%)
PFHxS			
Mean (SD)	1.15 (1.69)	0.23 (0.23)	0.74 (1.34)
Median [Min, Max]	0.60 [0.01, 13.00]	0.17 [0.04, 2.40]	0.33 [0.01, 13.00]
Non-quantified, n (%)	5 (2.7%)	1 (0.7%)	6 (1.8%)
PFOA			
Mean (SD)	1.35 (0.90)	0.73 (0.40)	1.07 (0.78)
Median [Min, Max]	1.16 [0.17, 8.05]	0.63 [0.19, 3.20]	0.85 [0.17, 8.05]
Non-quantified, n (%)	4 (2.2%)	1 (0.7%)	5 (1.5%)
PFOS			
Mean (SD)	4.09 (2.43)	2.44 (1.65)	3.35 (2.27)
Median [Min, Max]	3.54 [0.17, 15.05]	1.9 [0.52, 12.00]	2.69 [0.17, 15.05]
Non-quantified, n (%)	4 (2.2%)	2 (1.3%)	6 (1.8%)
PFNA			
Mean (SD)	0.58 (0.35)	0.46 (0.33)	0.52 (0.35)
Median [Min, Max]	0.51 [0.02, 3.16]	0.37 [0.08, 2.30]	0.44 [0.02, 3.16]
Non-quantified, n (%)	4 (2.2%)	1 (0.7%)	5 (1.5%)
PFUnDA			
Mean (SD)	0.23 (0.13)	0.11 (0.08)	0.18 (0.12)
Median [Min, Max]	0.21 [0.04, 0.68]	0.09 [0.03, 0.55]	0.14 [0.03, 0.68]
Non-quantified, n (%)	13 (7%)	6 (4.1%)	19 (5.7%)
PFDA			
Mean (SD)	0.28 (0.15)	0.18 (0.15)	0.24 (0.16)
Median [Min, Max]	0.26 [0.06, 1.09]	0.14 [0.03, 1.00]	0.19 [0.03, 1.09]
Non-quantified, n (%)	5 (2.7%)	1 (0.7%)	6 (1.8%)

[a] Molecular sum of MEHP, MECPP, MEHHP, MEOHP with the unit of mol/mL.

analysis on CP and LB demonstrated little evidence for associations despite an overall inverse trend (Fig. S3).

4. Discussion

This study is one of the first to evaluate the impact of mixtures of known and suspected EDCs on ovarian function and subsequent fertility in women. We observed significant associations of high levels of DEHP metabolites and methylparaben, and possibly PFUnDA and PFOA, with lower OSI, suggesting that these chemicals may interfere with ovarian sensitivity in women.

The potential link between exposure to EDCs and fertility has been the focus of several recent studies and literature reviews (Mínguez-Alarcón and Gaskins, 2017; Björvang et al., 2022; Kahn et al., 2020; Björvang and Damdimopoulou, 2020; Karwacka et al., 2019). Although male factors, like semen quality and sperm DNA damage, have been thoroughly studied and commonly used as a marker for male fecundity in chemical risk assessment (Meeker et al., 2010; Pant et al., 2008; European Food Safety Authority, 2018; EFSA Panel on Food Contact Materials et al., 2019), limited information is however available

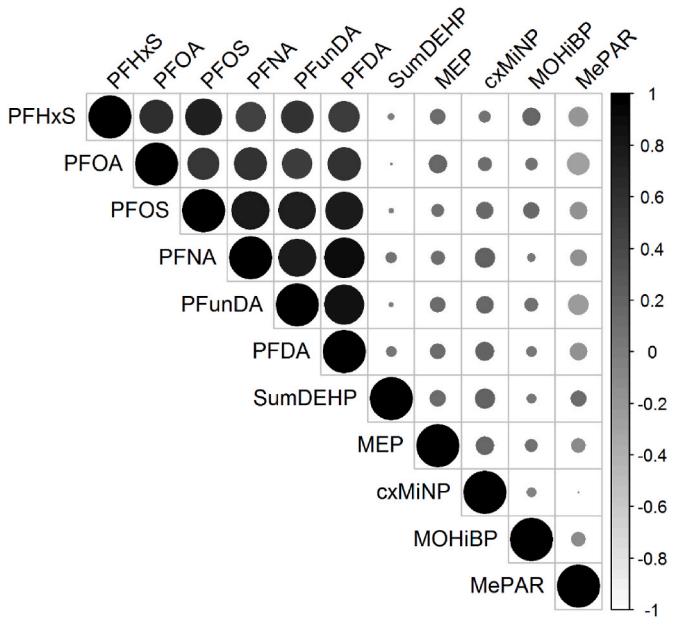

Fig. 1. Correlation of chemical measurements for the compounds detected with >LOQ in >90% of samples. Darker color and larger sizes indicated higher correlation coefficients.

on the potential effects of EDCs on female fertility and fecundity. A recent review on EDCs and female fecundity highlighted some potential causes of previous inconsistency, including heterogeneity in study population selection, exposure assessment, and co-exposure to other chemicals (Mínguez-Alarcón and Gaskins, 2017). Similarly, a review paper specifically focusing on IVF outcomes concluded that the evidence supporting an association between EDC exposures and ovarian reserve or IVF outcomes in humans remains limited (Karwacka et al., 2019).

Several studies conducted on women visiting fertility clinics have documented associations between higher levels of EDC exposure and less favorable success measures, such as lower rates of CP or LB (Björvang et al., 2022; Karwacka et al., 2019; Mínguez-Alarcón et al., 2015, 2019). Nevertheless, solely focusing on these clinical outcomes might fail to distinguish between paternal and maternal causes of infertility. Little attention has been paid to the associations between EDCs and female hormonal indicators, thus limiting our understanding of the potential effects of these widespread chemicals on women's fecundity and fertility (Karwacka et al., 2019). Here, we focus on OSI to specifically consider the associations of EDC exposure to indicators of ovarian function. OSI has been shown to be a good biomarker of female fertility and predictor of success in ART. As a marker of ovarian responsiveness to exogenous gonadotrophin stimulation, OSI is a predictor of IVF/ICSI outcome that has been shown to be superior to baseline FSH or AMH in predicting pregnancy as well as the total number of oocytes (Vaegter et al., 2017, 2019; Weghofer et al., 2020; Revelli et al., 2020). OSI is a measurement of ovarian competence

Table 3
Associations of phthalates and parabens concentrations with Ovarian Sensitivity Index, evaluated with individual regression models for each chemical.[a].

Plasticizer concentrations	Combined population (n = 333)[b]			Sweden (n = 185)[c]			Estonia (n = 148)		
	n	Beta	95% CI	n	Beta	95% CI	n	Beta	95% CI
ΣDEHP									
Q1 [0.0003, 0.0031]	82	Ref	–	51	Ref	–	31	Ref	–
Q2 (0.0031, 0.0039]	82	−0.05	(-0.16, 0.07)	51	−0.08	(-0.23, 0.06)	31	−0.05	(-0.24, 0.14)
Q3 (0.0039, 0.0049]	82	−0.05	(-0.16, 0.07)	40	−0.09	(-0.24, 0.07)	42	−0.05	(-0.22, 0.13)
Q4 (0.0049, 0.0144]	82	−0.07	(-0.19, 0.04)	39	**−0.21**	**(-0.38–0.05)**	43	−0.01	(-0.18, 0.16)
MEP									
Q1 [0.29, 0.51]	82	Ref	–	23	Ref	–	59	Ref	–
Q2 (0.51, 0.71]	88	−0.06	(-0.18, 0.06)	59	0.06	(-0.13, 0.25)	29	−0.10	(-0.27, 0.07)
Q3 (0.71, 0.98]	73	−0.02	(-0.14, 0.11)	56	0.09	(-0.11, 0.28)	17	−0.08	(-0.29, 0.12)
Q4 (0.98, 12.00]	78	−0.02	(-0.14, 0.10)	43	0.03	(-0.18, 0.24)	35	−0.01	(-0.17, 0.15)
cxMiNP									
Q1 [0.10, 0.30]	85	Ref	–	42	Ref	–	43	Ref	–
Q2 (0.30, 0.58]	79	0.00	(-0.11, 0.12)	46	−0.03	(-0.20, 0.13)	44	−0.05	(-0.22, 0.11)
Q3 (0.58, 1.80]	81	−0.04	(-0.15, 0.07)	54	−0.07	(-0.23, 0.09)	27	−0.15	(-0.33, 0.03)
Q4 (1.8, 70.00]	80	−0.08	(-0.19, 0.04)	40	−0.06	(-0.23, 0.11)	40	−0.13	(-0.30, 0.03)
MOHiBP									
Q1 [0.02, 0.03]	87	Ref	–	28	Ref	–	59	Ref	–
Q2 (0.03, 0.05]	120	0.05	(-0.06, 0.15)	65	−0.05	(-0.21, 0.12)	55	0.10	(-0.04, 0.24)
Q3 (0.05, 0.06]	40	0.11	(-0.03, 0.25)	26	0.02	(-0.18, 0.22)	14	0.17	(-0.05, 0.39)
Q4 (0.06, 0.41]	70	0.01	(-0.11, 0.13)	53	−0.15	(-0.33, 0.02)	17	0.13	(-0.08, 0.34)
Methylparaben									
Q1 [0.13, 5.30]	84	Ref	–	71	Ref	–	13	Ref	–
Q2 (5.30, 30.00]	80	0.03	(-0.09, 0.14)	60	0.06	(-0.07, 0.19)	20	−0.11	(-0.37, 0.15)
Q3 (30.00, 71.00]	83	−0.11	(-0.23, 0.02)	14	−0.02	(-0.25, 0.20)	69	**−0.22**	**(-0.44, -0.01)**
Q4 (71.00, 6000.00]	80	−0.05	(-0.17, 0.07)	35	−0.08	(-0.24, 0.08)	45	−0.08	(-0.31, 0.14)
cxMiNCH[d]									
Q1 [0.01, 0.04]		Ref	–	70	Ref	–	\	Ref	–
Q2 (0.04, 0.08]	\	\	\	33	0.03	(-0.12, 0.19)	\	\	
Q3 (0.08, 0.15]	\	\	\	29	−0.11	(-0.28, 0.06)	\	\	
Q4 (0.15, 16.00]	\	\	\	43	−0.14	(-0.30, 0.01)	\	\	
Propylparaben[e]									
Q1 [0.07,0.35]		Ref	–	\	Ref	–	6	Ref	–
Q2 (0.35, 6.90]	\	\	\	\	\	\	32	−0.22	(-0.54, 0.11)
Q3 (6.90, 15.00]	\	\	\	\	\	\	55	−0.23	(-0.55, 0.08)
Q4 (15.00, 110.00]	\	\	\	\	\	\	54	−0.13	(-0.44, 0.19)

[a] Adjusted for age, BMI, parity, and previous IVF.
[b] Further adjusted for cohort to account for structural differences between cohorts.
[c] Further adjusted for infertility duration, smoking, fatty fish consumption, and PCP use.
[d] High detection only in the Swedish population.
[e] High detection only in the Estonian population.

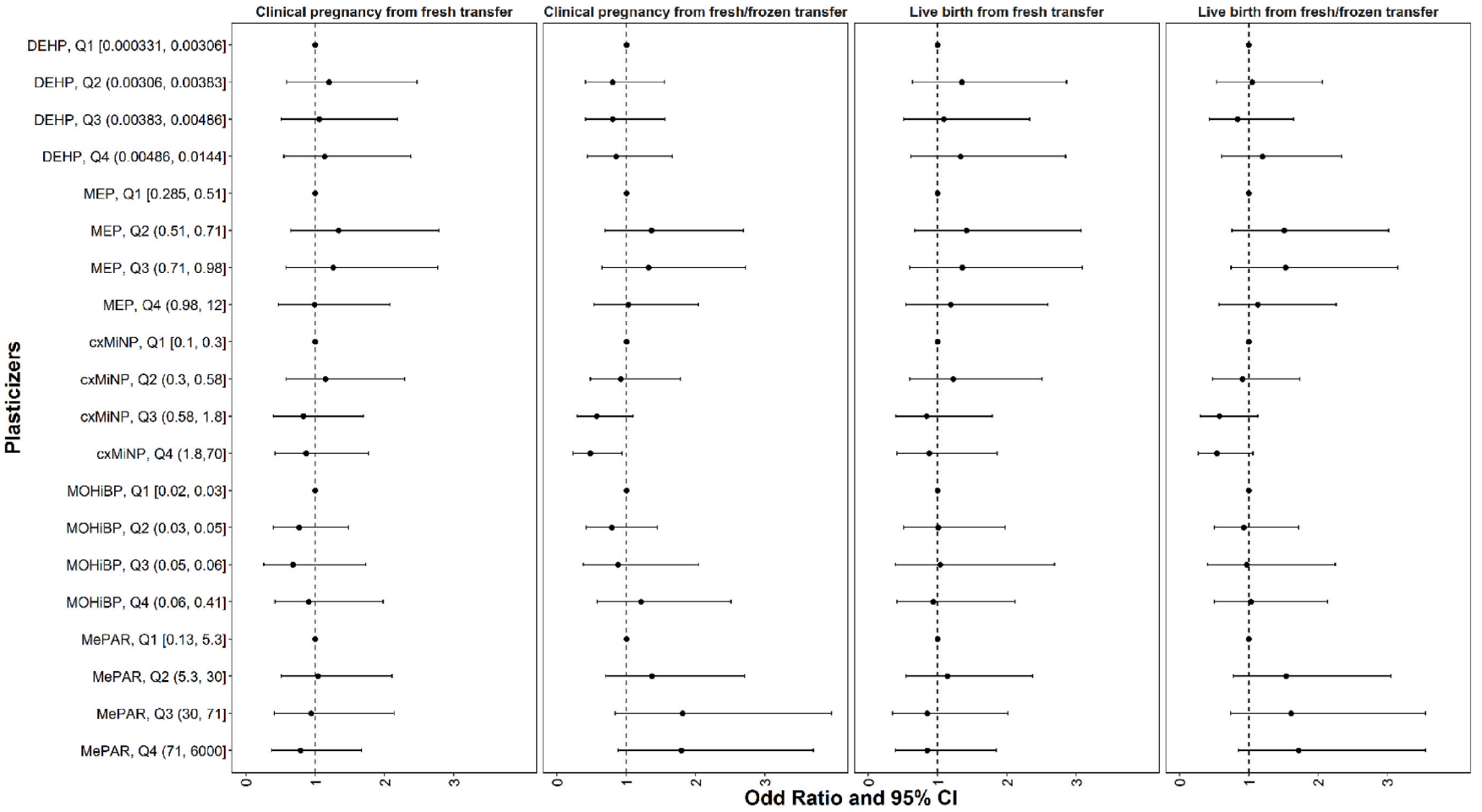

Fig. 2. Associations of phthalates and parabens concentrations with clinical pregnancy and live birth, assessed with individual logistic regression models adjusted for age, BMI, parity, previous IVF, and cohort, in the overall sample of Swedish and Estonian women. Chemicals were evaluated as categorical variables by quartiles of their distribution. Quartiles ranges are reported in parentheses. Results were based on observed 106 CP from fresh, 155 CP from fresh/frozen, 93 LB from fresh, and 135 LB from fresh/frozen transfers.

Table 4
Associations of PFAS concentrations with Ovarian Sensitivity Index, evaluated with a single regression model, mutually adjusting for all chemicals.[a].

PFAS concentrations	Combined population (n = 333)[b]			Sweden (n = 185)[c]			Estonia (n = 148)		
	n	Beta	95% CI	n	Beta	95% CI	n	Beta	95% CI
PFHxS									
Q1 [0.01, 0.17]	84	Ref	–	11	Ref	–	73	Ref	–
Q2 (0.17, 0.33]	80	−0.07	(-0.22, 0.09)	33	−0.03	(-0.34, 0.28)	47	−0.06	(-0.26, 0.13)
Q3 (0.33, 0.69]	81	0.00	(-0.17, 0.18)	58	0.00	(-0.31, 0.31)	23	0.05	(-0.18, 0.29)
Q4 (0.69, 13]	82	−0.002	(-0.20, 0.20)	78	0.02	(-0.30, 0.35)	4	0.10	(-0.32, 0.51)
PFOA									
Q1 [0.17, 0.60]	82	Ref	–	17	Ref	–	65	Ref	–
Q2 (0.60, 0.85]	83	0.10	(-0.03, 0.23)	38	0.14	(-0.12, 0.40)	45	0.10	(-0.05, 0.26)
Q3 (0.85, 1.33]	81	0.10	(-0.05, 0.25)	55	0.24	(-0.00, 0.49)	26	−0.01	(-0.22, 0.21)
Q4 (1.33, 8.05]	82	0.03	(-0.15, 0.21)	71	0.15	(-0.13, 0.43)	11	**−0.31**	**(-0.61, -0.01)**
PFOS									
Q1 [0.17, 1.84]	82	Ref	–	15	Ref	–	67	Ref	–
Q2 (1.84, 2.69]	82	0.01	(-0.16, 0.19)	44	0.17	(-0.15, 0.49)	38	−0.01	(-0.23, 0.22)
Q3 (2.69, 4.34]	81	−0.10	(-0.30, 0.10)	58	0.03	(-0.30, 0.36)	23	−0.05	(-0.34, 0.24)
Q4 (4.34, 15.10]	82	0.03	(-0.20, 0.26)	64	0.23	(-0.12, 0.58)	18	−0.24	(-0.66, 0.17)
PFNA									
Q1 [0.02, 0.30]	82	Ref	–	24	Ref	–	58	Ref	–
Q2 (0.3, 0.44]	82	0.15	(-0.01, 0.31)	47	0.15	(-0.12, 0.42)	35	0.14	(-0.09, 0.36)
Q3 (0.44, 0.64]	82	0.05	(-0.16, 0.26)	51	0.10	(-0.25, 0.45)	31	−0.02	(-0.30, 0.27)
Q4 (0.64, 3.16]	82	0.11	(-0.14, 0.35)	59	0.08	(-0.31, 0.47)	23	0.23	(-0.18, 0.64)
PFUnDA									
Q1 [0.03, 0.08]	82	Ref	–	15	Ref	–	67	Ref	–
Q2 (0.08, 0.14]	75	**−0.16**	**(-0.31, -0.02)**	33	0.05	(-0.21, 0.31)	42	**−0.27**	**(-0.45, -0.08)**
Q3 (0.14, 0.25]	78	−0.05	(-0.23, 0.13)	51	0.03	(-0.25, 0.31)	27	−0.16	(-0.41, 0.09)
Q4 (0.25, 0.68]	79	−0.08	(-0.31, 0.15)	73	0.06	(-0.26, 0.39)	6	−0.33	(-0.77, 0.10)
PFDA									
Q1 [0.03, 0.13]	82	Ref	–	18	Ref	–	64	Ref	–
Q2 (0.13, 0.19]	82	−0.10	(-0.26, 0.06)	43	0.04	(-0.28, 0.36)	39	−0.03	(-0.25, 0.18)
Q3 (0.19, 0.31]	81	−0.03	(-0.24, 0.18)	54	0.17	(-0.22, 0.55)	27	0.05	(-0.24, 0.33)
Q4 (0.31, 1.09]	82	−0.11	(-0.36, 0.13)	65	−0.03	(-0.44, 0.38)	17	0.30	(-0.07, 0.67)

[a] Adjusted for age, BMI, parity, and previous IVF.
[b] Further adjusted for cohort to account for structural differences between cohorts.
[c] Further adjusted for infertility duration, smoking, fatty fish consumption, and PCP use.

reflecting the response to exogenous FSH stimulation. The reason for its introduction in reproductive endocrinology was the observation that although the total number of oocytes retrieved at ovum pick-up correlates to pregnancy rate, these correlations are much stronger if account is also taken of the dose of FSH and human menopausal gonadotropin (hMG) given (Huber et al., 2013). Physiologically, this factor is thus describing a stimulus-response observation, and the most proper way to measure it is as the number of oocytes retrieved divided by the total dose of FSH/hMG used. The high prediction potential likely relates to the fact that OSI reflects not ony the number of oocytes left in the reserve, but also functional aspects of ovaries, ultimately associated with the likelihood of retrieving euploid oocytes at ovum pick-up (Huber et al., 2013; Weghofer et al., 2020). Based on these data, we selected OSI as an appropriate outcome variable to reflect the female side specifically in ART.

Potential EDCs that we evaluated include phthalates, parabens, and PFAS, which can interfere with the endocrine system through a variety of complex biological mechanisms thus acting as disruptors of the endocrine system and potentially affecting biological systems related to reproductive health (La Merrill et al., 2020). Despite the growing evidence supporting a potential endocrine-disrupting the role of phthalates, parabens, and PFAS, very few chemicals are currently classified as EDCs by the EU. Svingen et al., 2022 Our results suggested the presence of inverse associations between some specific chemicals concentrations and OSI. We observed associations for phthalates, with a significant signal for DEHP metabolites among Swedish women, and parabens, with a significant signal for methylparaben among Estonian women. Both these results were robust to a set of sensitivity analyses. Higher molar sum of DEHP metabolites was previously associated with lower bAFC (Messerlian et al., 2016), and a lower probability of CP or LB following ART (Souter et al., 2013; Hauser et al., 2016). The current study suggested an inverse association between the molar sum of DEHP and OSI, which is consistent with most of the previous literature and strengthens the evidence on the role of this group of metabolites in the development of adverse reproductive outcomes (Panagiotou et al., 2021). Several mechanisms of action have been hypothesized to explain the potential association between DEHP and fertility as documented in animal studies, including the disruption of ovarian functioning and inhibiting the growth of antral follicles through reduced 17-beta oestradiol (E2) production (Panagiotou et al., 2021; Craig et al., 2014; Gupta et al., 2010; Hannon et al., 2014; Lovekamp-Swan and Davis, 2003). Interestingly, DEHP did not associate with CP or LB. The chemicals were measured only in the follicular fluid, and represent a snapshot of a time when oocytes were picked up. Follow-up studies need to assess potential associations between serum/urine DEHP and CP/LB during embryo transfer and early pregnancy. Previous studies have also reported associations of higher paraben concentration with lower rates of LB and poorer embryo quality (Dodge et al., 2015; Sabatini et al., 2011), while another study reported null associations (Mínguez-Alarcón et al., 2016). Mechanisms of actions for parabens are less clear and include oestrogenic activities such as their ability to bind with both oestrogen receptor ER-α and ER-β (Gomez et al., 2005; Okubo et al., 2001). Gonadotropin sensitivity is another indicator strictly related to OSI, which should also be further investigated (Björvang et al., 2022; Biasoni et al., 2011).

Evidence on PFAS and human fertility outcomes are sparse, and a recent review identified only two studies that documented either null associations or associations with higher androgen levels (Björvang and Damdimopoulou, 2020; Petro et al., 2014; Heffernan et al., 2018). This illustrates the complexity of assessing fertility in women and highlights the importance of studying large cohorts with multiple well-defined outcomes to identify sensitive endpoints for endocrine disruption (van Duursen, 2020). In contrast to phthalates and parabens, where we mostly observed inverse associations robust to different modeling and sensitivity analyses, our results for PFASs were less robust, and we only

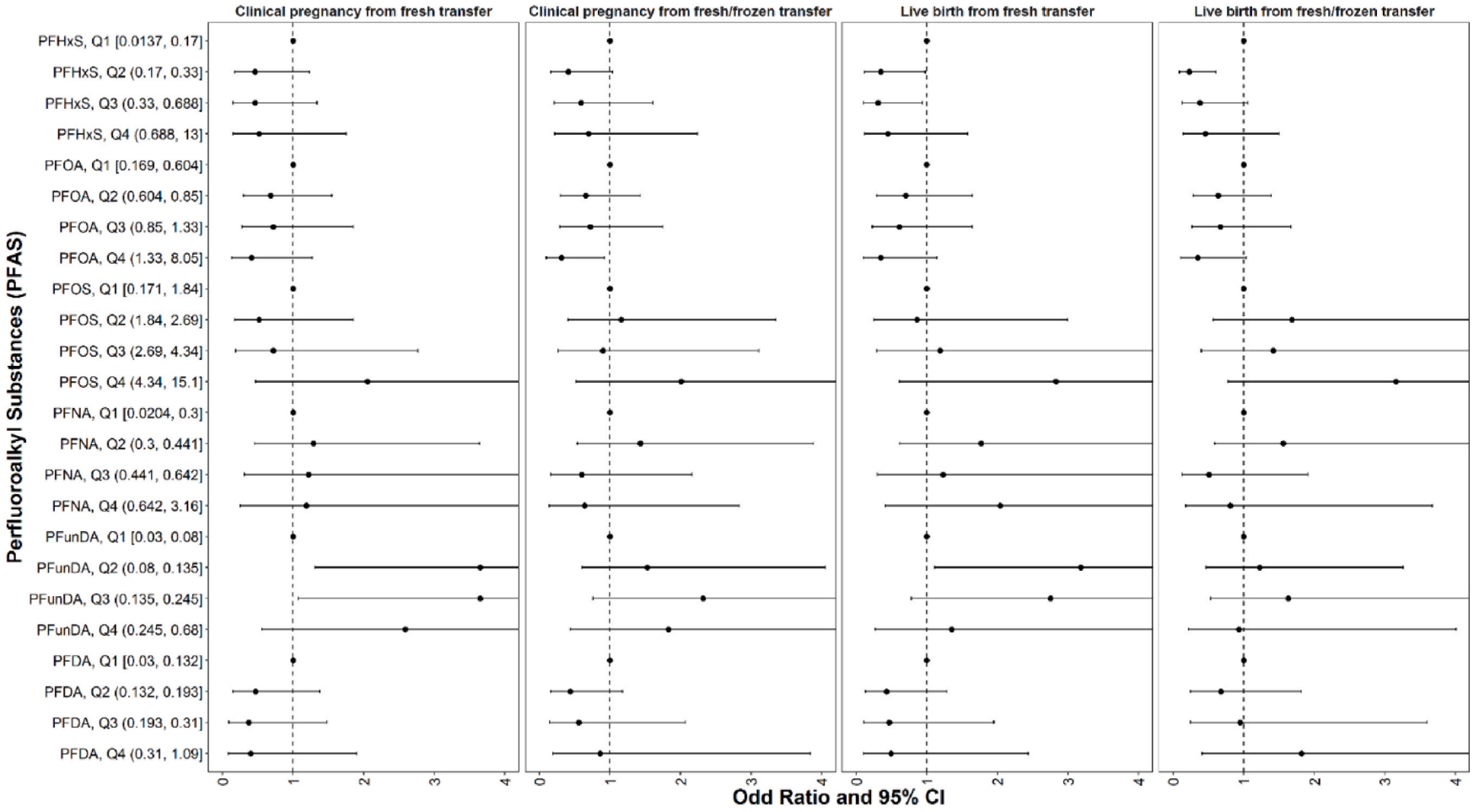

Fig. 3. Association of PFAS concentrations with clinical pregnancy and live birth, assessed with one multiple regression model adjusted for age, BMI, parity, previous IVF, and cohort, in a combined cohort of Swedish and Estonian women. PFAS were evaluated as categorical variables by quartiles of their distribution. Quartiles ranges are reported in parentheses. Results were based on observed 106 CP from fresh, 155 CP from fresh/frozen, 93 LB from fresh, and 135 LB from fresh/frozen transfers.

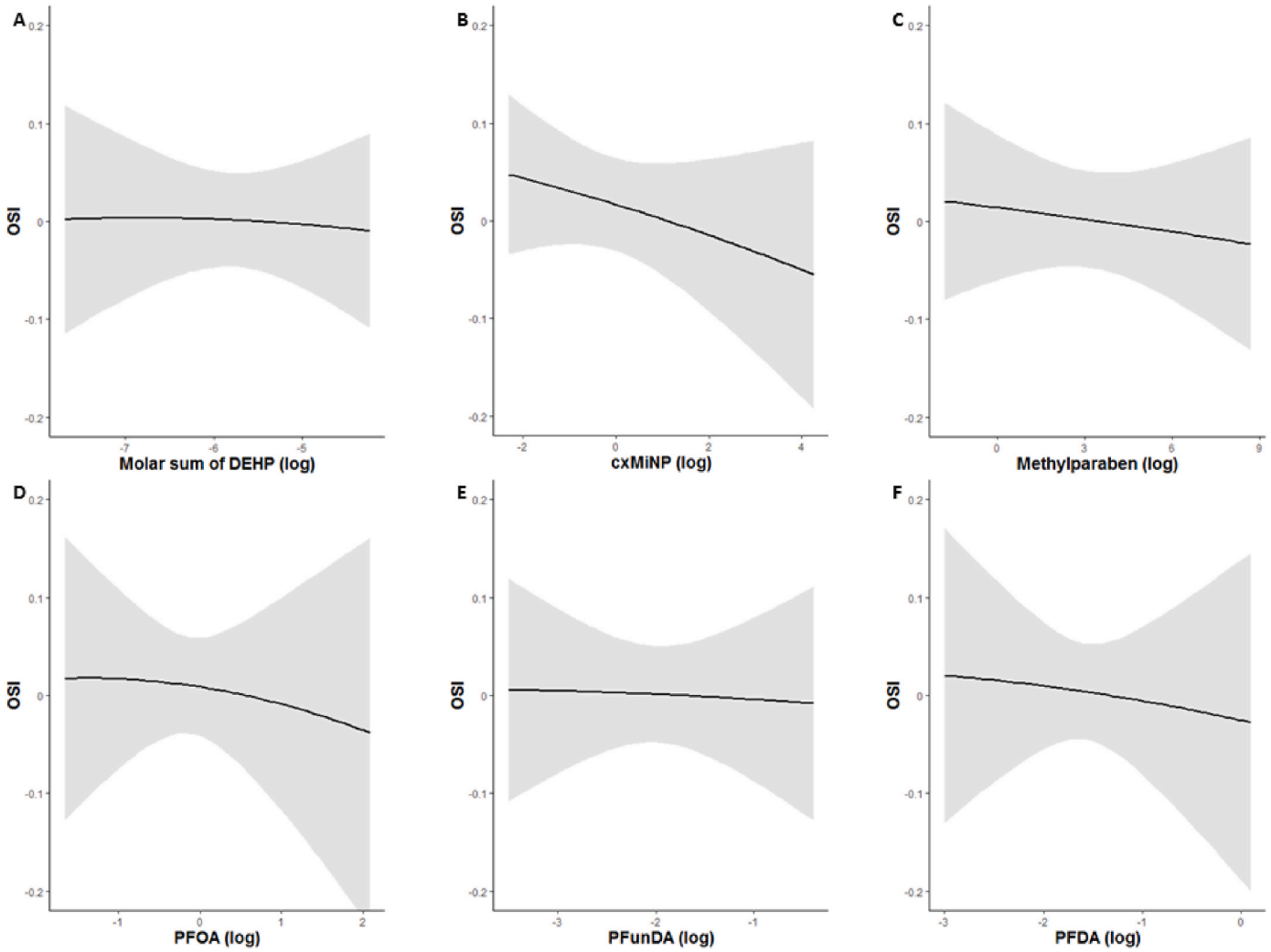

Fig. 4. Dose-response associations of DEHP (A), cxMiNP (B), methylparaben (C), PFOA (D), PFUnDA (E), and PFDA (F) with Ovarian Sensitivity Index, adjusted for age, BMI, parity, previous IVF, estimated using hierarchical Bayesian Kernel Machine Regression in a subsample with complete information (n = 283). The grey areas indicate 95% credible intervals.

observed a signal for PFUnDA and PFOA in multivariable models where associations were found both with OSI and CP/LB. These results were likely affected by high levels of collinearity and were not confirmed by the BKMR analysis. While the complex correlation structure reported among PFAS compounds could certainly play a role, these results also suggest that the association between these compounds and fertility outcomes is less consistent. An earlier study on the Swedish cohort that focused on chemicals discovered associations of PFAS with bAFC and embryo quality, but not with OSI, CP, or LB (Björvang et al., 2022). Identification of critical effects to various types of chemicals in humans remains a high-priority task, and our data suggest that within the fertility domain, multiple targets may be included with varying sensitivity to chemical exposures.

We also evaluated the associations of individual chemicals and chemical mixtures with clinical outcomes such as CP and LB. These analyses, however, were severely hampered by the low number of CP and LB available in our populations. Specifically, we only had data on 155 clinical pregnancies and 135 live births, which limited the statistical power of the logistic regression analysis. Larger cohorts or a longer-follow up time might enable higher power. Although we still observed some significant associations of higher PFHxS and cxMiNP concentrations with lower odds of CP and LB, we did not detect associations with common chemicals that have been previously associated with CP and LB, such as DEHP (Mínguez-Alarcón and Gaskins, 2017). As such, these results should be interpreted with caution.

Our study attempted to address some of the methodological challenges described by the previous literature and, as such, has several strengths (Mínguez-Alarcón and Gaskins, 2017). Firstly, we conducted our analysis within two separate cohorts, which improved the generalizability of our findings. Results on phthalates and parabens were robust in the stratified analysis as well as when using methods that account for between-cohorts variability. Secondly, to our knowledge, this is one of the first studies to investigate associations between EDCs and OSI. Future studies should investigate the role of OSI and other female fertility measures within the causal pathways leading from EDCs exposure to clinical outcomes, and evaluate the role of these early pregnancy female indicators in the associations between chemical exposures and clinical outcomes. Moreover, our results also inform toxicological studies about a potential key role of OSI, which could be evaluated as an endpoint in animal studies to test its connection to EDCs and its ability to independently predict female fecundity (van Duursen, 2020). Thirdly, we measured chemical concentrations from follicular fluids, which might enrich the ability to account for direct exposure to the maturing follicle (Hallberg et al., 2021). Previous studies, including a recent assessment in the Swedish cohort, have shown that levels of exposure assessed in the blood correlated with those assessed from the follicular fluid (rho 0.64–0.99) (Björvang et al., 2022). Finally, we conducted a thorough examination of EDC exposures as chemical mixtures. While it

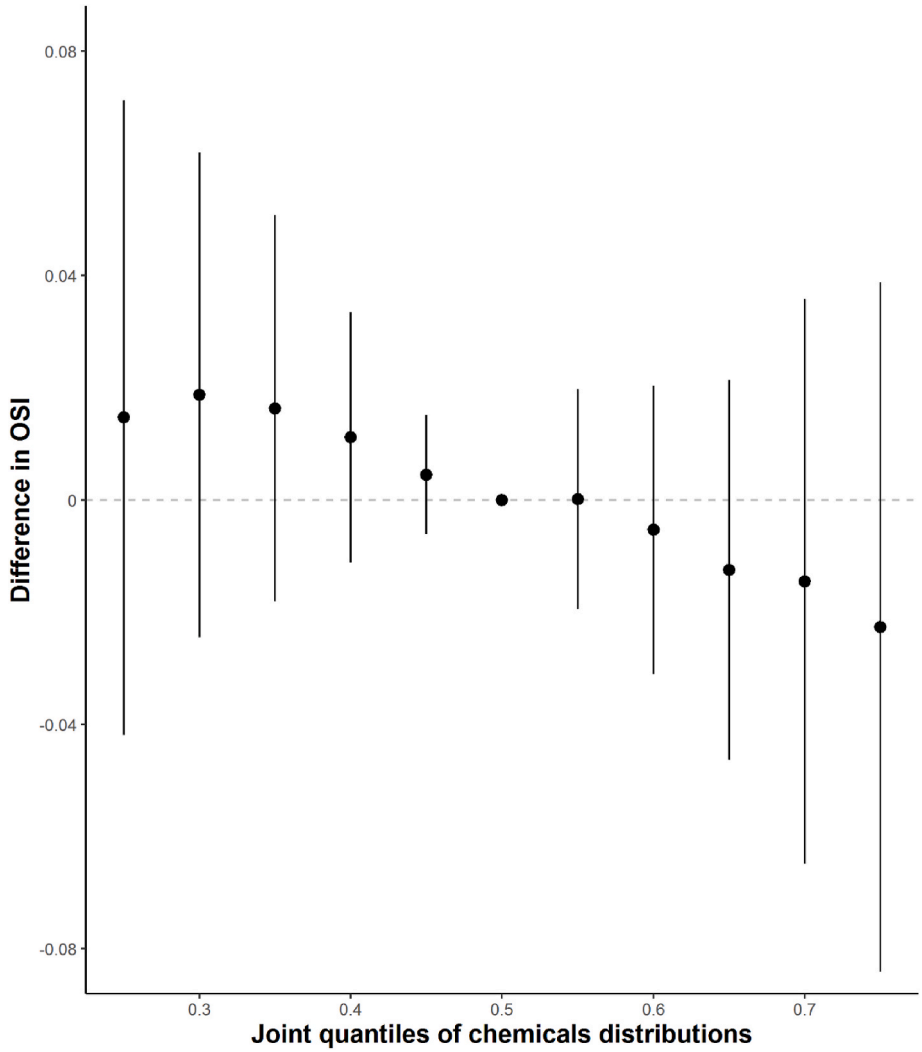

Fig. 5. Overall mixture effect of chemicals on Ovarian Sensitivity Index (OSI), adjusted for age, BMI, parity, previous IVF, estimated using hierarchical Bayesian Kernel Machine Regression in a subsample with complete information (n = 283). For each quintile (x-axis) point estimates and credible intervals represent the change in OSI when each chemical is set to that quintile of their distributions, as compared to when they are all set at their median.

is recognized that environmental exposures are present in the world as a complex mixture, most epidemiologic studies fail to integrate the presence of co-exposures into the analyses, which has been recognized as one of the potential causes of inconsistency among previous studies (Mínguez-Alarcón and Gaskins, 2017). The switch to a mixture framework has been repeatedly indicated as a priority in the field (Dominici et al., 2010; Taylor et al., 2016; Kortenkamp, 2007; Drakvik et al., 2020), and several analytical techniques to achieve this goal have been described and presented (Stafoggia et al., 2017; Hamra and Buckley, 2018; Gibson et al., 2019). In this study, we examined the structure of the mixture in terms of correlation clusters and described the ability of regression methods to characterize the data. To allow further flexibility and relax regression assumptions, we also sought to strengthen our findings with BKMR. Despite the relatively small sample size, this method allowed for estimating the overall effect of the chemical mixture, suggesting the presence of an inverse cumulative trend.

Due to the relatively small sample size, the power of our analysis was limited, especially with regards to the binary outcomes of CP and LB. Because of the small sample size, together with the high number of evaluated models, we cannot exclude that our results might arise due to a chance component. Nevertheless, results from the mixture modeling, which incorporates all chemicals in a single statistical model, mostly confirmed results obtained from regression models. In addition, the finding of an inverse association between the overall mixture and OSI strengthens the interpretation of our chemical-specific results. Nevertheless, BKMR does not consider absolute values of exposure levels or toxicology of each chemical and does not therefore provide straightforward clinical interpretations. The relatively small sample size also prevented us from conducting stratified analysis to evaluate whether associations differ by relevant factors such as the cause of infertility. Another limitation is that, while PFAS are persistent chemicals, phthalates and parabens are quickly metabolized and their levels in biological samples are known to fluctuate. Therefore, the phthalates and parabens levels likely only reflect recent exposure rather than the typical levels during follicle growth and oocyte maturation, which takes approximately half a year in humans. Finally, while the combination of two cohorts provides additional strengths, a drawback was that we could not adjust for the whole set of confounders in all analyses. Despite evaluating several potential confounders and defining criteria for covariates inclusion, residual confounding might still be present and hamper the generalizability of our findings, which should be validated in other populations.

In conclusion, this study provided additional evidence supporting the presence of an inverse association between DEHP metabolites and female fertility and identifying additional chemicals such as methylparaben, and possibly PFUnDA and PFOA, that can be involved in the

biological processes causing female infertility via disruption of ovarian function. By accounting for the complexity of the chemical exposures and by directly evaluating a critical marker of female infertility, this study adds robust evidence to the literature to support the adverse effects of EDCs on reproductive health, with potential implications for public health interventions and recommendations.

Credit author statement

Andrea Bellavia: Formal analysis, Data Curation, Writing – Original Draft, Review and Editing; Runyu Zou: Formal analysis, Data Curation, Writing – Original Draft, Visualization; Richelle D. Björvang: Investigation, Data Curation, Writing – Original Draft; Kristine Roos: Formal analysis, Investigation, Resources, Data Curation, Writing – Original Draft; Ylva Sjunnesson: Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition; Ida Hallberg: Methodology, Investigation; Jan Holte: Resources; Anne Pikki: Investigation; Virissa Lenters: Methodology, Writing – Reviewing & Editing; Lützen Portengen: Methodology; Jacco Koekkoek: Investigation, Formal Analysis; Marja Lamoree: Validation, Supervision, Writing – Review and Editing; Majorie Van Duursen: Conceptualization, Project administration, Funding acquisition, Writing – Original Draft, Review and Editing; Roel Vermeulen: Conceptualization, Writing – Reviewing & Editing, Funding Acquisition; Andres Salumets: Conceptualization, Writing – Reviewing & Editing, Funding Acquisition; Agne Velthut-Meikas: Conceptualization, Resources, Data Curation, Writing – Original Draft, Review and Editing, Supervision, Funding Acquisition; Pauliina Damdimopoulou: Conceptualization, Resources, Writing – Original Draft, Review and Editing, Supervision, Project Administration, Funding Acquisition. All authors read, reviewed and approved the final manuscript.

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.

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Appendix A. Supplementary data

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