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Associations between organophosphate flame retardants metabolites in follicular fluid and reproductive outcomes among women undergoing IVF/ICSI treatment in Southwest China

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Abstract

Background Previous studies suggest organophosphate flame retardants (OPFRs) negatively affect fertility, but limited research explores their metabolites in follicular fluid and reproductive outcomes.

Objectives To investigate the associations between concentrations of OPFRs metabolites in follicular fluid and the outcomes of in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) among women undergoing treatment.

Methods Women who underwent IVF/ICSI treatment at the Reproductive Center of West China Second University Hospital, Sichuan University, China, from 2017 to 2020 were recruited. The levels of seven OPFRs metabolites were quantified in follicular fluid collected on the day of oocyte retrieval. Reproductive outcomes were assessed, including key IVF/ICSI outcomes.

Results This study included 401 women. After adjusting for relevant confounders, elevated concentrations of BBOEP (β = -0.08, 95% CI: -0.12 to 0 0.05), BEHP (β = -0.11, 95% CI: -0.17 to 0.05), DnBP (β = -0.23, 95% CI: -0.37 to 0.08), and DPhP (β = -0.12, 95% CI: -0.18 to 0.06) in follicular fluid were inversely associated with the number of good embryos on day 3. Elevated BEHP concentrations were negatively associated with the total number of oocytes (β = -0.04, 95% CI: -0.07 to 0.01). In comparison with the lowest tertile, the highest tertile of DnBP was associated with a 42% reduction in biochemical pregnancy (p-trend = 0.05). Furthermore, the BKMR models revealed inverse associations between OPFRs metabolites mixtures and the number of good embryos.

Conclusion Findings suggest OPFRs may negatively affect IVF/ICSI outcomes, warranting further study on environmental impacts on fertility.

Keywords Organophosphate flame retardants, Follicular fluid, Reproductive outcomes

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Background

Infertility, a condition marked by the inability to achieve pregnancy following twelve months or more of regular unprotected intercourse, impacts millions worldwide, exerting significant effects on individuals, their families, and the larger community. Roughly one in six individuals of reproductive age are estimated to experience infertility during their lifetime [1]. The reasons behind infertility are complex and not fully understood, with environmental factors serving as potential contributors to the condition [2]. The past few decades have witnessed remarkable advancements in science and industrialization, providing numerous conveniences in daily life. However, along with these advancements, there has been an increasing frequency and intensity of human exposure to chemical substances, raising concerns about potential health risks. Environmental endocrine-disrupting chemicals (EDCs) constitute a group of exogenous chemicals or chemical mixtures that can enter the human body through various environmental media, disrupting the synthesis, secretion, transport, metabolism, and action of endogenous hormones, thereby affecting normal reproductive function and embryo development [3]. Currently, more than 1400 chemical species have been identified as environmental EDCs, among which organophosphate flame retardants (OPFRs), a class of chemicals widely used to enhance the fire resistance of various materials, have emerged as a new class of pollutants of significant concern with studies linking OPFRs exposure to alterations in neurodevelopment, thyroid function, and reproductive toxicity [4–9].

The extensive utilization of OPFRs results in their widespread presence across various environmental mediums, including air, water, sediment, and dust [10, 11]. OPFRs can infiltrate the human body through multiple avenues, such as dietary ingestion, inhalation, dust consumption and dermal contact [12–15]. Commonly studied mediums for assessing human OPFRs exposure comprise blood, breast milk, hair, urine and nails [16]. Monitoring human exposure to OPFRs presents challenges due to the short half-lives (ranging from hours to days) of OPFRs in biological organisms [10]. Following entry into the body, OPFRs undergo degradation as metabolites via five distinct metabolic pathways in humans, including dealkylation, hydroxylation, oxidative dechlorination, oxidation, and conjugation [17]. Due to their rapid metabolization, the metabolites are used as biomarkers for analysis and assessing internal exposure to OPFRs, which are frequently detected in the urine [18]. In vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) has emerged as an effective therapy for patients with infertility. Previous epidemiological studies have found that OPFRs metabolites levels in urine of women undergoing IVF/ICSI are positively correlated with low fertilization rates, low embryo implantation rates, high pregnancy loss, and other adverse outcomes [19–21]. However, studies investigating the impact of OPFRs metabolites levels in follicular fluid on IVF/ICSI outcomes remain limited. Follicular fluid, which serves as the nurturing medium surrounding developing oocytes, plays a pivotal role in providing essential nutrients, growth factors, and hormones crucial for follicular growth and oocyte quality [22]. Therefore, follicular fluid may offer a more appropriate and biologically relevant matrix for assessing ovarian exposure to OPFRs.

The objective of the present study was to: (1) Report the levels of OPFRs metabolites in follicular fluid in females of childbearing age from southwest part of China. (2) Investigate whether levels of OPFRs metabolites measured directly in follicular fluid were associated with reproductive outcomes among Women Undergoing IVF/ICSI treatment in China.

Methods

Study design and population

This study was established within a reproductive cohort conducted in Chengdu, China. Briefly, women who underwent IVF/ICSI treatment at the Reproductive Center of West China Second University Hospital, Sichuan University, China, from January 2017 to December 2020 were recruited. Biological samples, including follicular fluid, along with clinical characteristics and reproductive outcomes, were collected during the treatment process. Among the participants, 408 individuals underwent IVF/ICSI treatment and provided follicular fluid samples. We excluded one woman due to missing reproductive outcome data and six women because their embryos underwent further testing for preimplantation genetic testing.

Information on demographic and clinical factors was gathered through medical records at the time of enrollment. BMI was calculated by dividing weight by the square of height. Education was categorized into "college and below" or "bachelor's degree and above," while ethnicity was distinguished between Han and other Chinese minorities. Hormone levels, including AMH, E2, FSH, and LH levels on days 2–3, were assessed. Infertility duration was defined as the length of time (in years) spent attempting to conceive. Infertility type was categorized as primary or secondary, with primary infertility indicating the patient that has never achieved a pregnancy, while secondary infertility refers to the patient who has previously conceived but is struggling to conceive again. Infertility factors were classified into female, male, mixed, or unexplained categories. Female factors include disorders affecting the female reproductive system, such as pelvic issues and ovulation disorders, while male factors involve conditions affecting the male genital system, such as dysspermia and sexual dysfunction. Cases where both male and female factors are present within one couple are

classified as mixed factors. Infertility without a definitive diagnosis is categorized as unexplained infertility. Each participant signed an informed consent form upon enrollment. The research protocol was approved by the Ethics Committee of West China Second University Hospital, Sichuan University of Science and Technology.

IVF/ICSI protocol and reproductive outcomes

The controlled ovarian stimulation treatment included agonist, antagonist protocols, as well as other approaches such as minimal ovarian stimulation and natural cycle. Upon reaching ≥ 18 mm, 1–3 leading follicles were triggered with 5,000–10,000 IU of HCG (Chorionic Gonadotrophin for Injection, Lizhu Group, Zhuhai, China) or 0.2 mg of Triptorelin (Triptorelin Acetate for Injection, Ipsen Pharma Biotech, Paris, France). Follicular aspiration was performed under sedation 35–36 h later. Fertilization was achieved through either conventional IVF or ICSI, based on semen analysis and prior fertilization results. Embryos were cultured in vitro at the cleavage or blastocyst stage and cryopreserved using vitrification. Luteal support for fresh cycles commenced on the day of oocyte retrieval. Frozen embryo transfer was conducted via either a natural or medicated cycle, tailored to patient characteristics and clinician preferences.

The reproductive outcomes, encompassing IVF/ICSI parameters, including oocyte number, mature oocyte number rate, fertilization rate, good embryo number, as well as pregnancy outcomes including biochemical pregnancy, clinical pregnancy, and live birth rates, were extracted from the electronic medical records. A mature oocyte was characterized as one exhibiting the first polar body indicative of its readiness for fertilization. Fertilization rate denoted the proportion of zygotes with two pronuclei formed after fertilization, relative to the total oocyte count. Good embryos were defined as those reach the eight-cell stage with less than 20% fragmentation [23]. Biochemical pregnancy was established by a human chorionic gonadotropin level exceeding 30 mIU/mL measured 14 days post-embryo transfer. Clinical pregnancy was confirmed by the presence of an intrauterine gestational sac observed on ultrasound examination after 6–7 weeks of gestation [24]. Live birth signified the delivery of a newborn demonstrating vital signs such as breathing, heartbeat, or observable voluntary muscle movements [25].

Chemicals and reagents

Seven OPFRs metabolites (Supplemental Table 1), including Bis(2-chloroethyl) phosphate (BCEP), Bis(1-chloro-2-propyl) phosphate (BCIPP), Bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), Diphenyl phosphate (DPhP), Bis(2-butoxyethyl) phosphate (BBOEP), Di-n-butyl phosphate (DnBP), and Bis(2-ethylhexyl) phosphate (BEHP), and its corresponding internal standard (BCEP-d8, BCIPP-d12, BDCIPP-d10, DPhP-d10, BBOEP-d8, BEHP-d34 and DBP-d18) were purchased from Toronto Research Chemicals (Canada). Acetonitrile, n-hexane, toluene, acetone, ethyl acetate, methylene chloride and cyclohexane used in this experiment are all chromatographic grade. Methanol and acetone were obtained from Fisher Scientific (Hanover Park, IL). Ethyl acetate and dichloromethane were provided by Honeywell (Muskegon, MI, USA). N-hexane were purchased from CNW Technologies GmbH (Germany). Ultrapure water was prepared by a MilliQ ultrapure system (Millipore).

Sample collection and analysis

Samples collected from the women during their egg retrieval were subjected to centrifugation at 4000 rpm for 10 min. The supernatant was then transferred to a new tube and stored at -80 °C. Analysis of the samples was conducted using a modified version of a previously published method [26]. After completely thawing the follicular fluid sample and mixing it at room temperature, transfer a measured volume of 1 mL of the follicular fluid into a polypropylene (PP) tube with a capacity of 15 mL. Add 5 µL of an internal standard mixture with a concentration of 1 µg/mL and let it stand overnight at rest. Subsequently, incorporate 5 mL of ethyl acetate, vortex for 20 min, and then centrifuge at 4000 rpm for 20 min to obtain the supernatant. Repeat the extraction process three times, pooling the supernatants from each round. Evaporate the combined supernatants to dryness using nitrogen gas and reconstitute the residue in 0.5 mL of n-hexane. The extract was loaded onto a Sep-Pak cartridge (3 cm/ 200 mg, Waters, Milford, MA, USA). The cartridge was vacuum-dried for 20 min and then eluted with 3 mL of mAcetone: n-hexane (1:1). The eluate was evaporated almost to dryness under a stream of nitrogen, and the residue was reconstituted with 100 µL 50% methanol aqueous solution.

The analysis of OPFRs metabolite was performed using UHPLC-MS/MS (4500 Qtrap, SCIEX, Redwood City, USA). The working conditions can be found in Supplemental Table 2. Multiple reaction monitoring (MRM) parameters of the OPFRs metabolites are listed in Supplemental Table 3. The chromatographic separation of 7 OPFRs metabolites are summarized in Supplemental Fig. 1. The average recoveries of OPFRs metabolites ranged from 75.8 to 90.7%. During sample analysis, every 50 samples are analyzed, a group of samples with quality control is added, and the blank of the same steps is taken as the sample to check whether there is cross-contamination in the pretreatment process. Every 100 samples are analyzed, a group of standard curves is added again to check the reproducibility of the method, and the slope RSD ($n = 4$) of the standard curves is within 3%.

Statistical analysis

In the descriptive analysis, continuous variables were summarized using means and standard deviations, while categorical variables were presented as frequencies and percentages. Compounds with detection frequencies (DFs) greater than 50% were retained for further analysis. Non-detectable chemical values (< LOD) were replaced by LOD/2, and values between LOD and the Lower Limit of Quantification (LQD) were replaced by 2*LOD. Spearman correlation tests were used to assess correlations between OPFRs metabolites in follicular fluid. The OPFRs metabolites were categorized both as categorical variables (tertiles) and continuous variables, undergoing a natural logarithm transformation to account for their skewed distribution. To examine linear trends across tertiles of OPFRs metabolites, participants were assigned the median value in each tertile and modeled as a continuous variable. Coefficients and their 95% confidence intervals (CIs) were estimated in multivariable generalized linear regression models (GLM) to assess the associations of individual OPFRs metabolites in follicular fluid with IVF/ICSI parameters. The multivariable models included several covariates: age, BMI, AMH, education, COH protocols, infertility factors, and infertility type. For oocyte number and good embryo number, Poisson distribution and log link were used. For mature oocyte number rate and fertilization rate, binomial distribution and probit link were employed. To test the associations with reproduction outcomes, including biochemical pregnancy, clinical pregnancy, and live birth, GLM with a binomial distribution and logit link were adjusted for age, BMI, education, COH protocols, infertility factors, infertility type, number of embryos transferred, embryo type, fertilization method, and stage of embryo transferred. In all models, smoking status was not included as a confounder, given the very low prevalence of smokers in our sample. Two sensitivity analyses were performed. First, recognizing the considerable impact of age on IVF/ICSI outcomes, analyses were performed in different age layers (< 35 and ≥ 35). Second, we limited our analysis to couples with female factors to mitigate the influence of other reproductive factors on IVF/ICSI outcomes.

In addition to assessing individual exposure to OPFRs metabolites, we conducted Bayesian Kernel Machine Regression (BKMR) to evaluate the associations between mixtures of OPFRs metabolites and IVF/ICSI outcomes. We utilized the "bkmr" package (version 0.2.2) in R software, employing 10,000 iterations. Confounders, consistent with the analysis of individual OPFRs metabolite exposure, were adjusted in all models. For both the individual and mixure analyses, age and BMI were considered as continuous variables in the analysis. Ethnicity was excluded from consideration due to the limited representation of women from other Chinese minorities.

All statistical analyses were conducted using RStudio software (version 2021.09.1) or STATA 18.0 (Stata Corporation). A two-tailed $P < 0.05$ was considered statistically significant.

Results

Characteristics of the study participants

Table 1 presents the demographic and clinical characteristics of 401 women undergoing IVF/ICSI treatment. The mean age of participants was 31.92 years with a standard deviation of 3.76, and the mean BMI was 21.31 kg/m^2 with a standard deviation of 2.67. The majority of participants had a bachelor's degree or above (62.1%) and were of Han ethnicity (97.3%). The average AMH level was 4.63 ng/ml, and the mean E2, FSH, and LH levels on days 2–3 were 33.86 ng/ml, 6.06 mIU/ml, and 3.44 mIU/ml, respectively. Most participants had primary infertility (61.9%) and female factors were the most common cause of infertility (58.9%). The agonist protocol was more frequently used for controlled ovarian hyperstimulation (60.9%). Among the 401 participants, 2 individuals did not retrieve oocytes, and another 17 individuals did not obtain embryos. In terms of fertilization method, the majority underwent IVF (76.3%). Additionally, 59.1% of transferred embryos were fresh, with an average of 1.69 embryos transferred per cycle. Among outcomes, biochemical pregnancy occurred in 58.1% of cases, while clinical pregnancy and live birth rates were 54.4% and 47.9%, respectively.

OPFRs metabolites in follicular fluid

Seven OPFRs metabolites were quantified in follicular fluid samples, and Table 2 presents a summary of their concentration distributions with detection frequencies (DFs) exceeding 50%. BBOEP, BEHP and DPhP were detected with high frequency, 91.8%, 94.8% and 67.3% respectively, while DnBP was detected in all follicular fluid samples. Across percentiles, BBOEP exhibited concentrations ranging from < LOD to 13.22 ng/mL, while BEHP ranged from < LOD to 15.993 ng/mL. DnBP ranged from 0.062 to 1.488 ng/mL, and DPhP ranged from < LOD to 1.74 ng/mL. Notably, correlations between these metabolites were observed, with DPhP showing a strong positive correlation with BBOEP and DnBP, and BEHP exhibiting moderate negative correlations with BBOEP and DnBP, as revealed by Spearman correlation analysis (all p-values < 0.05, Table 3).

Associations of OPFRs metabolites in follicular fluid with IVF/ICSI outcomes

As shown in Table 4, significant associations between concentrations of individual OPFRs metabolites in follicular fluid and various IVF/ICSI parameters were observed. Elevated tertiles of BEHP metabolites were

Table 1 Characteristics of 401 women undergoing IVF/ICSI treatment

Characteristics	Mean ± SD or *n* (%)
Basic information	
Age (y)	31.92 ± 3.76
BMI (kg/m2)	21.31 ± 2.67
Education	
College and below	152 (37.9)
Bachelor's degree and above	249 (62.1)
Ethnicity	
Han	390 (97.3)
other Chinese minorities	11 (2.7)
Smoking status	6 (1.5)
Smoker	395 (98.5)
Non-smoker	
AMH (ng/ml)	4.63 ± 3.88
E2 level on D2-3 (ng/ml)	33.86 ± 14.44
FSH level on D2-3 (mIU/ml)	6.06 ± 3.09
LH level on D2-3 (mIU/ml)	3.44 ± 2.77
Cycle information	
Infertility duration	3.24 ± 2.34
Infertility type	
Primary infertility	248 (61.9)
Secondary infertility	153 (38.2)
Infertility factors	
Female factors	236 (58.9)
Male factors	70 (17.5)
Mixed factors	93 (23.2)
Unexplained	2 (0.5)
COH protocol	
Agonist	244 (60.9)
Antagonist	149 (37.2)
Others	8 (2.0)
Fertilization method (*n* = 399)	
IVF	306 (76.3)
ICSI	82 (20.5)
IVF + ICSI	11 (2.7)
Embryo type (*n* = 382)	
Fresh	237 (59.1)
Frozen	145 (36.2)
Number of embryos transferred (*n* = 382)	1.69 ± 0.56
Stage of embryo transferred (*n* = 382)	
Cleavage embryo	325 (81.1)
Blastocyst	57 (14.2)
Outcome	
Number of oocytes retrieved	10.41 ± 5.82
Mature oocytes rate (%)	
Fertilization rate (%)	58.5 ± 22.8
Number of good embryos	2.33 ± 2.60
Biochemical pregnancy	
No	168 (41.9)
Yes	233 (58.1)
Clinical pregnancy	
No	183 (45.6)
Yes	218 (54.4)
Live birth	
No	209 (52.1)
Yes	192 (47.9)

Note: SD, Standard Deviation; BMI, Body Mass Index; AMH, Anti-Müllerian Hormone; E2, Estradiol; FSH, Follicle Stimulating Hormone; LH, Luteinizing Hormone; COH, Controlled Ovarian Hyperstimulation; IVF, In Vitro Fertilization; ICSI, Intracytoplasmic Sperm Injection

significantly associated with a decrease in the number of retrieved oocytes, exhibiting a notable decreasing trend across tertiles ($p = 0.01$). Similarly, concentrations of BBOEP, DnBP, and DPhP showed a significant negative trend with the number of good embryos, with all p-values less than 0.001. Additionally, an increase in the logarithm of BEHP was negatively associated with the number of good embryos, with a coefficient of -0.11 (-0.17, -0.05). The analysis of associations between OPFRs metabolite concentrations in follicular fluid and pregnancy outcomes, as detailed in Table 5, largely revealed null associations. except, the highest tertile of DnBP was associated with a 42% reduction in biochemical pregnancy in comparison with the lowest tertile (p-trend = 0.05). In sensitive analysis, the primary findings remained unchanged and retained their significance after restricted the analysis to couples with female factors (Supplemental Tables 5–8). In the BKMR models, we observed that mixtures of OPFRs metabolites in follicular fluid were negatively associated with the number of good embryo (Fig. 1). The univariate exposure–response relationships were linear when other compounds were fixed at median values. No evidence of obvious interaction effects between the OPFRs metabolites.

Discussion

OPFRs are widely distributed in various environmental media and their reproductive toxicity has attracted increasing attention in recent years. However, research on the distribution of these chemicals in the human body, particularly in women undergoing IVF/ICSI remains relatively limited. Our study represents, to the best of our knowledge, the first report on the concentrations of OPFRs metabolites in follicular fluid and pregnancy outcomes. In the current study, we observed that four out of seven OPFRs metabolites were detected in more than 50.0% of follicular fluid samples, indicating these chemicals could pass through the blood–follicle barrier, enter the intra-ovarian environment, and directly affect the oocytes. The observed correlations between OPFRs metabolites suggest potential co-exposure or common sources of contamination.

In this study, we further investigated the associations between OPFRs metabolites in follicular fluid and reproductive outcomes among women undergoing IVF/ICSI

Table 2 The summary of OPFRs metabolites concentrations with detection frequencies (DFs) > 50% in follicular samples from females seeking for IVF/ICSI treatment

Analytes	DFs (%)	LOD (ng/ml)	Arithmetic mean (ng/mL)	Percentiles (ng/mL)						
				min	5th	25th	50th	75th	95th	max
BBOEP	91.8	0.048	1.872	< LOD	< LOD	0.096	0.933	3.152	5.933	13.220
BEHP	94.8	0.059	0.865	< LOD	< LOD	0.363	0.587	0.993	2.127	15.993
DnBP	100	0.084	0.200	0.062	0.076	0.139	0.176	0.214	0.322	1.488
DPhP	67.3	0.056	0.167	< LOD	< LOD	< LOD	0.130	0.232	0.405	1.740

Note: DFs, Detection frequencies; LOD, Limit of detection; BBOEP, Bis(2-butoxyethyl) phosphate; BEHP, Bis(2-ethylhexyl) phosphate; DnBP, Di-n-butyl phosphate; DPhP, Diphenyl phosphate

Table 3 The correlation among the concentrations of the OPFRs metabolites in the follicular samples ($n = 401$)

Correlation coefficient	BBOEP	BEHP	DnBP	DPhP
BBOEP	1			
BEHP	-0.333**	1		
DnBP	0.439**	-0.101*	1	
DPhP	0.740**	-0.186**	0.520**	1

Note: BBOEP, Bis(2-butoxyethyl) phosphate; BEHP, Bis(2-ethylhexyl) phosphate; DnBP, Di-n-butyl phosphate; DPhP, Diphenyl phosphate

* means the correlation is significant at the 0.05 level;

** means the correlation is significant at the 0.01 level

Table 4 Associations between OPFRs metabolite concentrations in follicular fluid and IVF/ICSI parameters ($n = 401$)

	Number of oocytes retrieved	Mature oocytes rate (%)	Fertilization rate (%)	Number of good embryos
OPFR metabolites	**Coefficient (95% CI)**	**Coefficient (95% CI)**	**Coefficient (95% CI)**	**Coefficient (95% CI)**
BBOEP				
Log	0 (-0.02, 0.02)	-0.01 (-0.09, 0.06)	-0.01 (-0.08, 0.06)	**-0.08 (-0.12, -0.05)**
Q1	ref	ref	ref	ref
Q2	-0.01 (-0.09, 0.07)	0.05 (-0.29, 0.38)	-0.02 (-0.33, 0.29)	**-0.24 (-0.40, -0.08)**
Q3	0.02 (-0.06, 0.10)	-0.07 (-0.41, 0.26)	-0.06 (-0.37, 0.25)	**-0.37 (-0.54, -0.21)**
p for trend	0.50	0.57	0.68	**< 0.001**
BEHP				
Log	**-0.04 (-0.07, -0.01)**	-0.02 (-0.16, 0.11)	0.01 (-0.12, 0.13)	**-0.11 (-0.17, -0.05)**
Q1	ref	ref	ref	ref
Q2	0.01 (-0.06, 0.08)	0.14 (-0.19, 0.48)	0 (-0.31, 0.30)	0.07 (-0.09, 0.22)
Q3	**-0.09 (-0.16, -0.01)**	0.15 (-0.18, 0.48)	-0.07 (-0.37, 0.24)	-0.1 (-0.26, 0.07)
p for trend	**0.01**	0.45	0.63	0.15
DnBP				
Log	-0.02 (-0.08, 0.05)	-0.02 (-0.31, 0.27)	-0.04 (-0.31, 0.22)	**-0.23 (-0.37, -0.08)**
Q1	ref	ref	ref	ref
Q2	-0.02 (-0.09, 0.06)	0.04 (-0.29, 0.38)	0.12 (-0.19, 0.42)	0.06 (-0.09, 0.21)
Q3	-0.03 (-0.10, 0.05)	0.12 (-0.21, 0.45)	-0.08 (-0.39, 0.22)	**-0.31 (-0.48, -0.15)**
p for trend	0.49	0.48	0.60	**< 0.001**
DPhP				
Log	0 (-0.03, 0.03)	-0.01 (-0.14, 0.12)	0 (-0.12, 0.12)	**-0.12 (-0.18, -0.06)**
Q1	ref	ref	ref	ref
Q2	-0.04 (-0.12, 0.03)	-0.05 (-0.37, 0.28)	-0.01 (-0.32, 0.30)	**-0.16 (-0.32, -0.01)**
Q3	0.03 (-0.05, 0.10)	-0.05 (-0.39, 0.28)	0.01 (-0.30, 0.32)	**-0.30 (-0.46, -0.14)**
p for trend	0.38	0.76	0.93	**< 0.001**

Note: Generalized linear regression models were adjusted for age, BMI, AMH, education, COH protocols, infertility factors, infertility type. IVF, In Vitro Fertilization; ICSI, Intracytoplasmic Sperm Injection; BBOEP, Bis(2-butoxyethyl) phosphate; BEHP, Bis(2-ethylhexyl) phosphate; DnBP, Di-n-butyl phosphate; DPhP, Diphenyl phosphate

treatment in China. Specifically, elevated BEHP concentrations were negatively associated with the total number of retrieved oocytes, suggesting a possible impact on ovarian function or follicular development. Additionally, higher levels of BBOEP, BEHP, DnBP, and DPhP in follicular fluid were inversely correlated with the number of good embryos on day 3, indicating potential impairment of embryo quality. We also observed a significant

Table 5 Associations between OPFRs metabolite concentrations in follicular fluid and pregnancy outcome ($n = 382$))

	Biochemical pregnancy	Clinical pregnancy	Live birth
OPFRs metabolites	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
BBOEP			
Log	0.97 (0.87, 1.10)	0.98 (0.87, 1.10)	0.98 (0.87, 1.10)
Q1	ref	ref	ref
Q2	1.04 (0.61, 1.79)	1.16 (0.68, 1.98)	1.32 (0.77, 2.26)
Q3	0.97 (0.57, 1.67)	0.96 (0.57, 1.64)	0.88 (0.52, 1.50)
p for trend	0.87	0.72	0.34
BEHP			
Log	0.86 (0.69, 1.08)	0.84 (0.68, 1.05)	0.89 (0.72, 1.11)
Q1	ref	ref	ref
Q2	0.85 (0.50, 1.45)	0.88 (0.52, 1.50)	0.98 (0.58, 1.67)
Q3	0.67 (0.39, 1.13)	0.73 (0.43, 1.23)	0.74 (0.43, 1.25)
p for trend	0.14	0.23	0.22
DnBP			
Log	0.59 (0.37, 0.9)	0.72 (0.45, 1.15)	0.70 (0.44, 1.13)
Q1	ref	ref	ref
Q2	0.88 (0.51, 1.52)	1.10 (0.65, 1.88)	1.14 (0.67, 1.93)
Q3	**0.58 (0.34, 0.99)**	0.69 (0.41, 1.16)	0.67 (0.39, 1.14)
p for trend	**0.046**	0.16	0.14
DPhP			
Log	0.92 (0.75, 1.14)	0.94 (0.77, 1.16)	0.98 (0.80, 1.21)
Q1	ref	ref	ref
Q2	1.07 (0.62, 1.83)	1.24 (0.73, 2.11)	1.20 (0.70, 2.04)
Q3	0.78 (0.46, 1.33)	0.82 (0.49, 1.40)	0.86 (0.50, 1.46)
p for trend	0.31	0.37	0.48

Note: Generalized linear regression models were adjusted for age, BMI, education, COH protocols, infertility factors, infertility type, number of embryos transferred, embryo type, fertilization method and Stage of embryo transferred. IVF, In Vitro Fertilization; ICSI, Intracytoplasmic Sperm Injection; BBOEP, Bis(2-butoxyethyl) phosphate; BEHP, Bis(2-ethylhexyl) phosphate; DnBP, Di-n-butyl phosphate; DPhP, Diphenyl phosphate

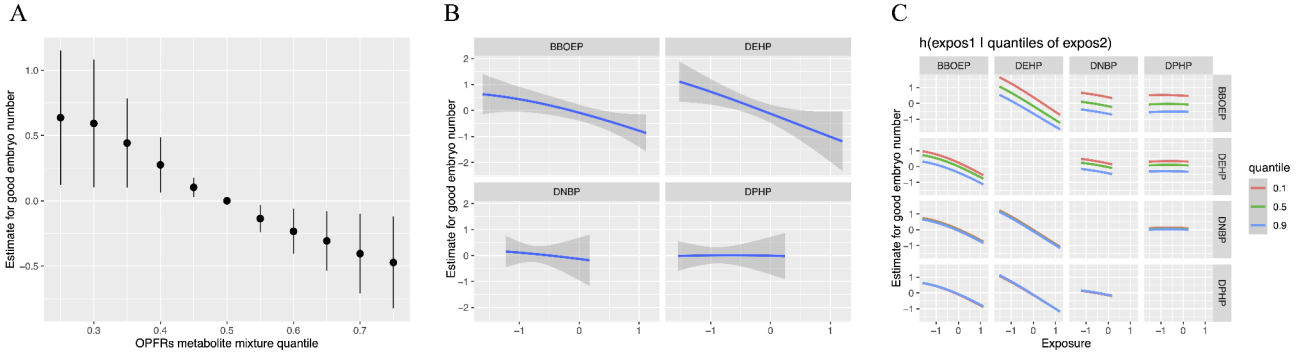

Fig. 1 **A**. Estimate effects (95% credible intervals) of the mixtures of OPFRs metabolites on good embryo number by BKMR models when log-transformed metabolite concentrations at particular percentiles were compared with all the OPFRs metabolites at their 50th percentile. **B**. Univariate exposure-response relationships and 95% credible intervals between log-transformed concentrations of individual OPFRs metabolite. **C**. Qualitative interaction assessment of OPFRs metabolites. The BKMR models were adjusted same as generalized linear regression

negative association between higher tertiles of DnBP and biochemical pregnancy rates underscoring the potential influence of OPFRs exposure on early pregnancy outcomes. The BKMR analysis further supported our findings, revealing a negative association between mixtures of OPFRs metabolites in follicular fluid and the number of good embryos. Infertility remains a significant public health concern globally, with an increasing number of couples resorting to IVF/ICSI to achieve conception. Our findings shed light on the potential impact of environmental exposures on IVF/ICSI success rates and highlight the importance of considering chemical pollutants in reproductive health research.

Although the precise mechanism remains not fully elucidated, emerging evidence and previous studies provide potential explanations. The observed associations

between OPFRs exposure and reproductive outcomes could be explained by the endocrine-disrupting effects of OPFRs on hormone levels and folliculogenesis. Epidemiologic study found that serum OPFRs levels in reproductive-age women are correlated with reproductive and thyroid hormone levels and are significantly associated with the risk of decreased ovarian reserve function [4]. Animal models have demonstrated that OPFRs exposure can affect the levels of estradiol and progesterone, thereby influencing folliculogenesis [27]. Moreover, in vitro experiments have shown that OPFRs can modulate the expression of estrogen, androgen, and glucocorticoid receptors, and interfere with the expression of steroid synthesis-related genes in adrenal cortical cells, testicular interstitial cells, and KGN ovarian granulosa cells [28–30]. Furthermore, OPFRs can act as endocrine disruptors by disrupting cholesterol homeostasis. Studies have indicated that OPFRs exposure is associated with a significant increase in lipid droplets in KGN ovarian granulosa cells and can induce lipid accumulation in ovarian stromal tissue in animals [31].

Despite the significance of our findings, several limitations should be acknowledged. Firstly, while utilizing FF as a biological matrix offers the advantage of directly measuring the exposure on ovary, it doesn't negate the possibility of exposure through other routes or potential contamination during sampling and handling. However, it's worth noting that if contamination had indeed occurred, one would expect consistent high concentration levels of these chemicals across all samples, which was not observed in the study. Moreover, the associations observed in this study could be influenced by other chemicals that were not specifically measured, as humans are constantly exposed to a multitude of chemicals simultaneously. Furthermore, FF samples provide only a snapshot of exposure during the specific follicular development stage when collected. Longitudinal studies should be done in future. Lastly, it is important to note that our study did not collect or analyze data on the concentrations of OPFRs in the semen of male partners. We are unable to determine whether OPFRs in semen influenced the reproductive outcomes observed. This limitation underscores the necessity for future studies to incorporate comprehensive analyses of OPFR concentrations in both male and female reproductive samples. Overall, while this study provides valuable insights, its findings must be interpreted within the context of these inherent limitations.

Conclusions

In conclusion, our findings highlight the significance of environmental exposures, such as OPFRs, as potential modifiable factors influencing IVF/ICSI outcomes. Addressing environmental contaminants through regulatory measures and lifestyle modifications may offer opportunities to improve IVF/ICSI success rates and mitigate the impact of environmental factors on reproductive health. Further research is needed to prove and elucidate the mechanisms underlying the observed associations.

Abbreviations

OPFRs	Organophosphate Flame Retardants
IVF/ICSI	In Vitro Fertilization/Intracytoplasmic Sperm Injection
EDCs	Endocrine-Disrupting Chemicals
BMI	Body Mass Index
AMH	Anti-Mullerian Hormone
E2	Estradiol
FSH	Follicle-Stimulating Hormone
LH	Luteinizing Hormone
COH	Controlled Ovarian Hyperstimulation
UHPLC-MS/MS	Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry
MRM	Multiple Reaction Monitoring
BCEP	Bis(2-chloroethyl) phosphate
BCIPP	Bis(1-chloro-2-propyl) phosphate
BDCIPP	Bis(1,3-dichloro-2-propyl) phosphate
DPhP	Diphenyl phosphate
BBOEP	Bis(2-butoxyethyl) phosphate
DnBP	Di-n-butyl phosphate
BEHP	Bis(2-ethylhexyl) phosphate
LOD	Limit of Detection
LQD	Lower Limit of Quantification
GLM	Generalized Linear Model
CI	Confidence Interval
BKMR	Bayesian Kernel Machine Regression
RCT	Randomized Controlled Trial
DFs	Detection Frequencies
p-trend	Trend Test p-value
RSD	Relative Standard Deviation

Supplementary Information

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Supplementary Material 1

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Author contributions

Xiaohong Li: investigation, methodology, data curation, writing—review and editing, and funding acquisition. Jiahui Qiu: formal analysis, validation, writing of original draft, and writing—review and editing. Zhiwei Gan: methodology, and writing—review and editing. Shangwei Li: investigation, data curation, resources, and writing—review and editing. Xun Zeng: project administration, conceptualization, supervision, writing—review and editing, funding acquisition.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The research protocol was approved by the Ethics Committee of West China Second University Hospital, Sichuan University of Science and Technology. The study was performed in accordance with the Declaration of Helsinki. Each participant signed an informed consent form upon enrollment.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

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