Follicular Fluid Perfluoroalkyl and Polyfluoroalkyl Substances Are Associated With Adverse ART Outcomes in Women With Poor Ovarian Reserve

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Research

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Abstract

Background

Previous evidence suggests that perfluoroalkyl and polyfluoroalkyl substances (PFASs) adversely affect ovarian function and female fecundity. However, the evidence remains insufficient to infer a direct relationship between PFAS exposure and adverse assisted reproductive technology (ART) outcomes. To fill this gap, we examined follicular fluid PFAS exposure and ART outcomes in patients with poor ovarian reserve (POR) in a prospective study.

Methods

In total, 147 women with POR were included. Eight PFASs were measured in follicular fluid (n=104) samples using simultaneous analysis by ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry. The PFAS contamination status of the patients' follicular fluid and the association between characteristics and ART outcomes were investigated by logistic regression.

Results

After adjustment for age and BMI, PFOA, PFNA, PFHxS and $\sum$ PFAS were strongly associated with a decreased probability of pregnancy (PFOA highest vs. lowest tertile: OR=1.95, 95% CI: 1.61, 2.38; PFNA highest vs. lowest tertile: OR= 3.0, 95% CI: 2.46, 3.68; PFHxS highest vs. lowest tertile: OR= 1.95, 95% CI: 1.61, 2.35; $\sum$ PFAS second vs. lowest tertile: OR=3.31, 95% CI: 2.74, 3.89). PFOS and PFUnDA were inversely associated with failed implantation. No relationships were noted between failed implantation and other PFAS analytes. The same result was obtained when using live birth as an outcome measure.

Conclusions

In women with POR, follicular fluid PFAS exposure may decrease the probability of clinical pregnancy and live birth.

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a class of endocrine-disrupting chemicals (EDCs) consisting of a 4–14 carbon chain decorated with several fluorine atoms and a charged functional group that have been widely used for decades in a variety of products, such as nonstick pans, water-resistant coatings and firefighting foams[1].

PFASs have recently received unparalleled attention because after years of phasing out, human biomonitoring surveys are still detecting PFASs in the majority of people worldwide. In the US National Biomonitoring Program, at least one type of PFAS was detected in the blood of nearly every person[2, 3].

Exposure to PFASs is known to be associated with a number of adverse health outcomes, such as the occurrence of diabetes[4], renal function[5], obesity and metabolic syndrome[6]. There is increasing evidence linking PFAS exposure to human reproductive health. Many epidemiological studies have suggested that exposure to PFASs is associated with a prolonged time to pregnancy (TTP) [7, 8, 9].

It has been reported that poor ovarian responders (PORs) to controlled ovarian hyperstimulation (COH) have lower pregnancy rates than normal responders, and evidence has demonstrated that the live birth rate per cycle is below 10%[10]. The main reason for the decreased pregnancy rates is generally associated with reduced oocyte quantity and quality. Accumulating evidence suggests that PFASs could adversely affect ovarian function by reducing ovarian hormone synthesis through activating peroxisome proliferator-activated receptors[11] and disrupting gap junction intercellular communication between oocytes and granulosa cells[12]. This evidence led us to consider whether maternal PFASs had effects on in vitro fertilization (IVF) outcomes among POR women.

Although most PFASs production is banned or restricted in developed countries[13], there has been considerable production and emission of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in China during the last two decades[14, 15], with much of the world production shifting to China in recent years. PFASs, especially PFOS and PFOA, have been detected in infertile women in Europe and the USA. However, information on the concentrations of PFASs in subfertile women is limited in China.

To the best of our knowledge, the effect of maternal PFAS concentrations on reproductive outcomes among POR women undergoing IVF remains unexplored. In this study, we aimed to evaluate whether follicular fluid PFAS levels are a prognostic marker of adverse reproductive outcomes following IVF treatment.

Methods

2.1. Patients

This study was approved by the Ethics Committee of the First People's Hospital of Yunnan Province (registration number: 2017YY138). All patients signed informed consent forms before being included in the study. A total of 147 patients diagnosed with POR were enrolled. The analysis included all autologous fresh or frozen embryo transfer (FET) cycles performed from March 2017 to September 2018, with livebirths followed up to December 2019. The definition of POR was in accordance with the Bologna criteria. If at least two of the following three features are present, POR can be diagnosed: 1) advanced maternal age (≥ 40 years) or any other risk factor for POR; 2) a previous POR (≤ 3 oocytes with a conventional stimulation protocol); and 3) an abnormal ovarian reserve test (antral follicle count $< 5-7$ follicles or anti-Müllerian hormone $< 0.5-1.1$ ng/ml). Patients were excluded from the study if they had severe male-factor infertility requiring intracytoplasmic sperm injection (ICSI) or were part of a couple with genetic disorders requiring preimplantation genetic testing. Cycles where no oocytes were retrieved were excluded from the final analysis.

Only women who achieved a clinical pregnancy rate per aspiration (CPR) or fully utilized all cryopreserved embryos during the study period were included to ensure the reliability of CPR estimates.

All women had a mild COH stimulation protocol: Letrozol (Furui) or clomiphene (Codal Synto) was given on day 3 of menstruation. When at least three follicles had reached 17 mm, human chorionic gonadotrophin (hCG, 5000-10,000 IU; Serono) was injected. Oocyte retrieval was performed by the transvaginal ultrasound-guided approach 34–36 h after HCG injection. Fertilization was confirmed by the presence of two pronuclei in the zygotes.

Embryo transfers of cryopreserved embryos were performed either in an hCG-triggered natural cycle using 5000 IU hCG on the day the leading follicle was ≥ 17 mm or, in the case of anovulatory infertility, in estradiol- and progesterone-substituted cycles (oral estradiol 2 mg three times daily from cycle Days 2–3 and Dydrogesterone (Abbott) added as luteal phase support 3 days prior to embryo transfer of cleavage stage frozen–thawed embryos and 6 days before transfer of vitrified-warmed blastocysts). Embryos were thawed on the transfer day. Up to two viable Day-3 embryos or one or two surviving blastocysts were transferred. In the hCG-triggered FET cycles, no luteal phase support was provided. Follicular fluid (FF) was collected only from the first puncture of one ovary. Samples were processed and stored at -20°C until analysis.

2.2 Reproductive outcomes

The primary outcome was the CPR, defined as the presence of an intrauterine gestational sac on transvaginal ultrasound. The secondary outcomes were the cumulative live birth per aspiration (CLBR), number of oocytes retrieved and fertilization rate. CLBR was defined as at least one liveborn baby at ≥ 20 weeks gestation resulting from an ART aspiration cycle, including all fresh and FETs resulting from the associated ovarian stimulation. Multiple deliveries from the same pregnancy were considered one live birth.

2.3 Chemical analysis

2.3.1. Sample preparation and analysis

Samples were analyzed for 8 PFASs as follows: PFOS, PFOA, heptafluorobutyric acid (PFBA), perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), perfluoroheptanoic acid (PFHpA), perfluoroundecanoic acid (PFUnDA) and perfluorodecanoic acid (PFDA), which included the two most widely studied PFASs, PFOA and PFOS, as well as those with different lengths of carbon chains (from 4 to 11 carbon atoms). Briefly, a 150- μL aliquot of each individual sample was transferred to an Eppendorf tube. After the addition of extract solution (methanol, containing isotopically labeled internal standard mixture), the samples were vortexed, centrifuged, and dried under a gentle nitrogen flow. Then, 100 μL of methanol:water = 7:3 was added to the tube, sonicated and centrifuged, and the clear supernatant was subjected to analysis.

UHPLC separation was carried out using an EXIONLC System (Sciex) equipped with a Waters ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm, Waters), and the injection volume was 5 μL. Mobile phase A was 3 mmol/L NH₄OH and 3 mmol/L ammonium acetate in water, and mobile phase B was acetonitrile. A SCIEX 6500 QTRAP + triple quadrupole mass spectrometer (Sciex) equipped with an IonDrive Turbo V electrospray ionization (ESI) interface was applied for assay development.

The MRM parameters for each of the targeted analytes were optimized using flow injection analysis by injecting the standard solutions of the individual analytes into the API source of the mass spectrometer. SCIEX Analyst Work Station Software (Version 1.6.3) and Sciex OS-Q software were employed for MRM data acquisition and processing.

2.4 Statistical methods

Data are given as the mean ± SD, the median and intertertile range or number (%), as appropriate. One-way ANOVA was used to analyze continuous data with a normal distribution. Chi-squared (x²) tests were used to compare categorical data, and when the expected frequency was less than 5, Fisher's exact test was used. Nonnormally distributed data were compared using the Wilcoxon rank-sum test. PFASs were investigated using the Spearman test. The odds ratio (OR) and 95% confidence interval (CI) were estimated by logistic regression. All statistical calculations were performed using SPSS 21. A *p* value less than 0.05 was considered statistically significant.

Results

3.1 Description of subjects

One hundred forty-seven POR women who underwent fresh or FET cycles performed from March 2017 to September 2018 at the First People's Hospital of Yunnan Province, PR China, were enrolled. Forty-three women were excluded because their IVF cycle was cancelled, and 250 aspiration cycles undertaken by 104 women were included in the final analysis. The 104 women had a mean age of 38.6 ± 3.2 years, the mean CPR was 18.9%, and the CLBR was 11.1%. The etiological factors of females participating in ART treatment in this study were 'tubal factor' (93.7%), endometriosis (3.1%) and unexplained reason (3.2%).

3.2 PFAS concentrations in ovarian FF

One hundred four follicular samples were analyzed for 8 PFASs. The distributions and limits of detection (LODs) of the PFAS concentrations in plasma are presented in Table 1. Seven PFASs, i.e., PFBA, PFHpA, PFOA, PFNA, PFOS, PFUnDA and PFDA, were detected in each sample. The detection frequencies of PFHxS was 98%. The concentrations from highest to lowest were PFBA (median 6.63, range 0.93–31.99 ng/mL), PFHpA (median 1.22, range 0.02–3.73 ng/mL), PFOA (median 1.13, range 0.83–4.17 ng/mL), PFOS (median 0.73, range 0.2–9.19 ng/mL), PFNA (median 0.51, range 0.18–1.21 ng/mL), PFUnDA (median 0.29, range 0.09–1.15 ng/mL), PFDA (median 0.23, range 0.07–0.65 ng/mL) and PFHxS (median 0.05, range 0.01–0.18 ng/mL). The FF ∑PFAS concentrations ranged from 2.8 to 38.1 ng/mL.

PFBA was the dominant compound in FF, with an average contribution of 54.9% $\sum$ PFAS. FF PFOS was significantly lower than that in previous studies[16, 17, 18].

Table 1
Distribution of follicular fluid PFAS in women undergoing IVF.

PFAS(ng/mL)	LOD	GM(SD)	Min	Median(IQR)	Max
PFBA	0.005	5.5(9.97)	0.93	6.63(1.54,18.99)	31.99
PFHpA	0.004	0.84(0.82)	0.02	1.22(0.95,1.54)	3.73
PFOA	0.005	1.08(0.61)	0.4	1.13(0.83,1.41)	4.17
PFNA	0.006	0.52(0.2)	0.18	0.51(0.41,0.74)	1.21
PFHxS	0.005	0.05(0.04)	<LOD	0.05(0.03,0.08)	0.18
PFOS	0.006	0.86(1.37)	0.2	0.73(0.55,1.21)	9.19
PFUnDA	0.007	0.27(0.19)	0.09	0.29(0.17,0.41)	1.15
PFDA	0.06	0.23(0.12)	0.07	0.23(0.15,0.3)	0.65
LOD, limit of detection; GM, geometric mean; SD, standard deviation; IQR, interquartile range.					

3.3 PFASs and ART outcome

To test the relationship between FF PFAS contamination status and ART outcome, we first divided subjects into two groups: the pregnant group (n = 46) and the nonpregnant group (n = 58). Lower FF PFOA [0.98 (0.82, 1.21) vs 1.15 (0.90, 1.53) ng/mL, $p = 0.034$], PFNA [0.46 (0.38, 0.67) vs 0.58 (0.47, 0.78) ng/mL, $p = 0.007$] and PFHxS [0.04 (0.03, 0.06) vs 0.05 (0.04, 0.08) ng/mL, $p = 0.034$] were found in women with ongoing pregnancy compared with women with implantation failure in the first complete cycle (Table 2).

Table 2
Clinical parameters and PFAS levels in pregnant and non-pregnant groups after ET

Characteristics	Pregnant group N = 46	Non-pregnant group N = 58	P
Age (years)	38(35,40)	40(37.8,42)	0.005
BMI(kg/m ²)	22.4(20.7,23.6)	22.2(21.7,23.6)	0.983
Duration of infertility(years)	5(2,6.75)	5(2.8,6)	0.507
AMH(ng/mL)	1.66(0.67,2.31)	0.88(0.65,1.87)	0.04
AFC	4(3,6)	3(2,5)	0.015
Oocytes(total)	3(2,4)	3(1.8,4)	0.593
Fertilization rate,%	71.4%	54.1%	0.018
PFBA(ng/mL)	8.52(1.56,19.14)	15.28(1.57,19.99)	0.861
PFHpA(ng/mL)	1.2(10.8,1.73)	1.22(0.99,1.52)	0.913
PFOA(ng/mL)	0.98(0.82,1.21)	1.15(0.90,1.53)	0.034
PFNA(ng/mL)	0.46(0.38,0.67)	0.58(0.47,0.78)	0.007
PFHxS(ng/mL)	0.04(0.03,0.06)	0.05(0.04,0.08)	0.034
PFOS(ng/mL)	0.87(0.63,1.26)	0.72(0.57,1.19)	0.88
PFUnDA (ng/mL)	0.3(0.18,0.38)	0.34(0.17,0.42)	0.694
PFDA(ng/mL)	0.24(0.16,0.29)	0.24(0.17,0.39)	0.304
∑PFAS(ng/mL)	14.01(4.93,23.34)	19.54(8.41,25.21)	0.239
∑ short-chain PFAS(ng/mL)	10.74(2.73,20.34)	16.3(4.61,21.21)	0.432
∑ long-chain PFAS(ng/mL)	2.83(2.14,3.44)	3(2.47,3.71)	0.162
Note: Values are Mean ± SD, median and interquartile range and % as indicated			
NS nonsignificant, which means P ≥ 0.05			
AFC antral follicle, BMI body mass index, AMH anti-Mullerian hormone			

Next, logistic regression was utilized to estimate the adjusted OR and 95% CIs. Based on previous research on PFASs[19], age and BMI were set as confounding factors and adjusted for in all of the regression models in our study.

In the crude analysis, elevated odds of failed implantation were observed for women whose FF PFNA, PFHxS and $\sum$ PFAS were in the highest tertiles (Table 3).

Table 3
Follicular fluid concentrations of PFASs and odds of clinical pregnancy rates

Perfluoro chemicals	Tertiles (ng/mL)	clinical pregnancy rates (%)	Cycles	Crude OR (95%CI)	Adjusted OR (95%CI)
PFBA	1st(0.93–1.59)	30.9	55	1 (reference)	1 (reference)
	2nd(> 1.59–16.73)	22.1	95	1.58(0.75–3.34)	0.53(0.17–1.68)
	3rd(17.33–31.99)	28	100	1.15(0.56–2.36)	0.32(0.09–1.08)
	<i>P</i> trend			<i>P</i> = 0.449	<i>P</i> = 0.185
PFHpA	1st(0.05–1.03)	29	62	1 (reference)	1 (reference)
	2nd(> 1.03–1.32)	22.6	124	1.4(0.7–2.8)	0.72(0.6–0.86)
	3rd(1.36–3.73)	31.3	64	0.9(0.42–1.93)	0.93(0.78–1.12)
	<i>P</i> trend			<i>P</i> = 0.384	<i>P</i> = 0.117
PFOA	1st(0.4–0.92)	30.8	91	1 (reference)	1 (reference)
	2nd(> 0.92–1.23)	26.8	115	1.26(0.69–2.32)	0.72(0.6–0.86)
	3rd(1.27–2.14)	18.2	44	2(0.83–4.85)	1.95(1.61–2.38)
	<i>P</i> trend			<i>P</i> = 0.303	<i>P</i> < 0.001*
PFNA	1st(0.18–0.45)	40	80	1 (reference)	1 (reference)
	2nd(> 0.45–0.68)	24.1	116	1.93(1.05–3.57)	1.15(0.96–1.34)
	3rd(0.71–1.21)	11.1	54	4.92(1.89–12.8)	3(2.46–3.68)
	<i>P</i> trend			<i>P</i> = 0.003*	<i>P</i> < 0.001*
PFHxS	1st(0.01–0.04)	37.1	89	1 (reference)	1 (reference)
	2nd(> 0.04–0.08)	27.5	91	1.56(0.83–2.92)	0.72(0.6–0.86)

OR, odds ratio; CI, confidence interval

P-value for test of trend across tertiles

Adjusted for age(continuous),BMI(continuous)

Perfluoro chemicals	Tertiles (ng/mL)	clinical pregnancy rates (%)	Cycles	Crude OR (95%CI)	Adjusted OR (95%CI)
	3rd(> 0.08–0.18)	11.4	70	4.57(1.95–10.71)	1.95(1.61–2.35)
	<i>P</i> trend			<i>P</i> = 0.002	<i>P</i> < 0.001*
PFOS	1st(0.34–0.69)	20	80	1 (reference)	1 (reference)
	2nd(0.69–1.21)	23.4	94	0.82(0.39–1.69)	0.4(0.23–0.49)
	3rd(1.21–9.19)	36.8	76	0.43(0.21–0.88)	0.53(0.44–0.64)
	<i>P</i> trend			<i>P</i> = 0.044*	<i>P</i> = 0.013
PFUnDA	1st(0.09–0.22)	15.9	88	1 (reference)	1 (reference)
	2nd(> 0.22–0.39)	44.9	78	0.23(0.1–0.48)	0.17(0.15–0.21)
	3rd(> 0.39–1.15)	28.6	84	0.47(0.23–0.99)	0.35(0.28–0.42)
	<i>P</i> trend			<i>P</i> < 0.001*	<i>P</i> < 0.001
PFDA	1st(0.07–0.22)	27.8	72	1 (reference)	1 (reference)
	2nd(> 0.22–0.3)	27.9	122	0.72(0.38–1.36)	0.43(0.36–0.52)
	3rd(0.33–0.65)	21.4	56	1.02(0.45–2.29)	0.92(0.76–1.1)
	<i>P</i> trend			<i>P</i> = 0.634	<i>P</i> = 0.11
Σ PFAS	1st(2.8–6.85)	42.6	61	1 (reference)	1 (reference)
	2nd(> 6.85–22.33)	27.9	111	1.61(0.85–3.08)	3.31(2.74–3.89)
	3rd(> 22.33–38.08)	23.1	78	2.48(1.19–5.15)	1.85(1.13–2.22)
	<i>P</i> trend			<i>P</i> = 0.05	<i>P</i> < 0.001
OR, odds ratio; CI, confidence interval					
P-value for test of trend across tertiles					
Adjusted for age(continuous),BMI(continuous)					

After adjustment for age and BMI, PFOA in the highest tertile was strongly associated with an increased risk of failed implantation (OR = 1.95, 95% CI: 1.61, 2.38). Moreover, the association of PFNA, PFHxS and $\sum$ PFAS with failed implantation persisted and was even stronger (PFNA highest vs. lowest tertile: OR = 3.00, 95% CI: 2.46, 3.68; PFHxS highest vs. lowest tertile: OR = 1.95, 95% CI: 1.61, 2.35; $\sum$ PFAS second vs. lowest tertile: OR = 3.31, 95% CI: 2.74, 3.89) (Table 3). In contrast, PFOS and PFUnDA were inversely associated with failed implantation. There were no relationships between failed implantation and other PFAS analytes.

Furthermore, when using live birth as an outcome measure, the same result was obtained. After adjustment for age and BMI, FF PFOA, PFNA, PFHxS and $\sum$ PFAS were strongly associated with increased risks of failed live birth (PFOA highest vs. lowest tertile: OR = 2.04, 95% CI: 1.63, 2.56; PFNA highest vs. lowest tertile: OR = 7.03, 95% CI: 5.35, 9.25; PFHxS highest vs. lowest tertile: OR = 2.04, 95% CI: 1.63, 2.56; $\sum$ PFAS second vs. lowest tertile: OR = 1.76, 95% CI: 1.43, 2.16) (Table 4). Inverse associations were observed for the highest levels of PFOS and PFUnDA.

Table 4
Follicular fluid concentrations of PFASs and odds of cumulative live birth per aspiration

Perfluorochemicals	Tertiles(ng/mL)	cumulative live birth per aspiration (%)	Cycles	Crude OR(95%CI)	Adjusted OR (95%CI)
PFBA	1st(0.93–1.59)	7.27	55	1 (reference)	1 (reference)
	2nd(> 1.59–16.73)	10.5	95	0.67(0.19–2.24)	0.53(0.17–1.68)
	3rd(17.33–31.99)	16	100	0.41(0.13,1.31)	0.32(0.19–1.08)
	<i>P</i> trend			<i>P</i> = 0.249	<i>P</i> = 0.185
PFHpA	1st(0.05–1.03)	6.45	62	1 (reference)	1 (reference)
	2nd(> 1.03–1.32)	12.9	124	0.47(0.15–1.45)	0.19(0.15–1.03)
	3rd(1.36–3.73)	15.6	64	0.37(0.11,1.26)	0.31(0.24–1.18)
	<i>P</i> trend			<i>P</i> = 0.276	<i>P</i> = 0.19
PFOA	1st(0.4–0.92)	10.9	91	1 (reference)	1 (reference)
	2nd(> 0.92–1.23)	13.9	115	0.53(0.33–1.78)	0.65(0.55–1.56)
	3rd(1.27–2.14)	9.09	44	1.24(0.37–4.18)	2.04(1.63–2.56)
	<i>P</i> trend			<i>P</i> = 0.66	<i>P</i> < 0.001*
PFNA	1st(0.18–0.45)	17.5	80	1 (reference)	1 (reference)
	2nd(> 0.45–0.68)	12.1	116	1.45(0.65–3.25)	1.04(0.87–1.25)
	3rd(0.71–1.21)	3.7	54	5.2(1.13–23.88)	7.03(5.35–9.25)
	<i>P</i> trend			<i>P</i> = 0.09	<i>P</i> < 0.001*

OR, odds ratio; CI, confidence interval

P-value for test of trend across tertiles

Adjusted for age(continuous),BMI(continuous)

Perfluorochemicals	Tertiles(ng/mL)	cumulative live birth per aspiration (%)	Cycles	Crude OR(95%CI)	Adjusted OR (95%CI)
PFHxS	1st(0.01–0.04)	14.6	89	1 (reference)	1 (reference)
	2nd(> 0.04–0.08)	14.9	91	1.03(0.45–2.36)	0.65(0.55–1.77)
	3rd(> 0.08–0.18)	5.71	70	2.82(0.89–9.08)	2.04(1.63–2.56)
	<i>P</i> trend			<i>P</i> = 0.184	<i>P</i> < 0.001*
PFOS	1st(0.34–0.69)	2.5	80	1 (reference)	1 (reference)
	2nd(0.69–1.21)	14.9	94	0.15(0.03–0.67)	0.07(0.05–0.16)
	3rd(1.21–9.19)	18.4	76	0.11(0.03–0.52)	0.05(0.03–0.06)
	<i>P</i> trend			<i>P</i> = 0.019*	<i>P</i> = 0.032
PFUnDA	1st(0.09–0.22)	2.3	88	1 (reference)	1 (reference)
	2nd(> 0.22–0.39)	17.9	78	0.11(0.02–0.48)	0.01(0.03–0.75)
	3rd(> 0.39–1.15)	16.7	84	0.12(0.03–0.53)	0.07(0.01–0.37)
	<i>P</i> trend			<i>P</i> = 0.013*	<i>P</i> = 0.007*
PFDA	1st(0.07–0.22)	13.9	72	1 (reference)	1 (reference)
	2nd(> 0.22–0.3)	8.19	122	1.81(0.71–4.58)	0.76(0.63–1.84)
	3rd(0.33–0.65)	17.9	56	0.74(0.29–1.93)	0.69(0.56–1.92)
	<i>P</i> trend			<i>P</i> = 0.165	<i>P</i> = 0.435
ΣPFAS	1st(2.8–6.85)	19.7	61	1 (reference)	1 (reference)

OR, odds ratio; CI, confidence interval

P-value for test of trend across tertiles

Adjusted for age(continuous),BMI(continuous)

Perfluorochemicals	Tertiles(ng/mL)	cumulative live birth per aspiration (%)	Cycles	Crude OR(95%CI)	Adjusted OR (95%CI)
	2nd(> 6.85–22.33)	5.4	111	4.29(1.52–12.09)	1.76(1.43–2.16)
	3rd(> 22.33–38.08)	15.4	78	1.35(0.59–3.25)	0.76(0.62–1.93)
	<i>P trend</i>			<i>P = 0.019*</i>	<i>P = 0.027*</i>
OR, odds ratio; CI, confidence interval					
P-value for test of trend across tertiles					
Adjusted for age(continuous),BMI(continuous)					

Last, a reduced fertilization rate was found in the highest tertile PFHxS and $\sum$ PFAS groups (PFHxS 43.9% vs 68.9%, $p \leq 0.001$; $\sum$ PFAS 52% vs 74.2%, $p \leq 0.001$).

Discussion

Our results indicate that follicular fluid PFAS exposure was associated with increased odds of low probability of fertilization, clinical pregnancy and live birth among POR women. These findings are consistent with prior literature that has evaluated the relationship between PFASs and female fecundity[7, 8, 9]. The majority of studies have suggested that PFASs affect human fecundity through a longer time to pregnancy (TTP)[7, 8, 9].

Unlike prior studies that followed a self-selected set of individuals who succeeded in conceiving, the research design in the present study focused on the impact of maternal PFAS exposure on ART outcomes. Our results provide evidence that elevated PFAS exposure reduced the probability of fertilization, pregnancy and live birth, which is partially in line with prior studies[16, 17]. For example, Governi L observed a significant negative correlation ($R = 0.75$; $p < 0.001$) between FF PFAS levels and the fertilization rate, and McCoy identified a negative relationship between FF PFDA and PFUnDA and blastocyst conversion rates[16, 17]. However, the researchers did not observe associations between PFAS exposure and pregnancy probability after IVF[16, 17, 18]. This was largely due to sample size constraints given the relative infrequency with which such outcomes occurred (the maximum sample size was 38). It is also possible that women with poor ovarian response are sensitive to reproductive toxic substances.

The underlying mechanisms of PFAS-induced reproductive toxicity in humans remain largely unknown, although some in vitro studies have reported that PFAS can directly interact with oocytes and granulosa cells and influence oocyte quality and survival[11, 12, 20]. For example, a recent study reported that $10 \mu\text{g mL}^{-1}$ PFNA for 22 h had a severe negative effect on blastocyst formation in bovine oocytes in vitro[11],

which could be attributed to the disturbance in lipid droplet distribution. Peroxisome proliferator-activated receptors (PPARs) are crucial for successful oocyte development and lipid metabolism [21, 22]. Previous experiments have shown that PFASs can bind and activate PPAR [23, 24], and the activity of human PPAR- α increased with increasing carbon backbone chain length [25]. It is plausible that PFASs may interfere with ovarian cell function and oocyte maturation by interacting with PPARs.

Furthermore, PFAS exposure could affect oocyte survival by altering cell–cell communication within a follicle. When treated with PFDA during porcine oocyte maturation *in vitro*, gap junction intercellular communication (GJIC) among cumulus cells and oocytes was disrupted, and the number of live oocytes and the percentage of matured oocytes decreased [20]. Similarly, Domínguez A's study found that PFOS interfered with GJIC in cumulus-oocyte complexes during the first hours of oocyte maturation [12].

On the other hand, our results highlighted the declining trend of a well-known long-chain PFAS compound (PFOS) in FF. More specifically, the concentration of PFOS in our study was more than four times lower than that in previous studies that recruited patients in the period of 2006–2011 and in 2015 [26, 27]. This finding confirms the decline in contamination with PFOS in China, which can be mainly attributed to the phasing out of PFOS-based products and the restriction of PFOS in industrial and consumer products by international organizations since 2009 [28, 29]. In contrast to the PFOS ban, PFOA is classified as a highly concerning substance [30] but has not yet been completely phased out worldwide. This may be an explanation as to why our PFOA follicular concentrations were quite comparable with previous studies [16, 26, 27].

To our knowledge, we are the first to report that PFBA was the PFAS found at the highest concentration in FF [16, 26, 27]. The PFAS contamination pattern in FF is very similar to that in Chinese drinking water [31]: PFBA is detected at the highest concentration, followed by PFHpA, PFOA, and PFOS. There are two reasons for this phenomenon. First, the increase in PFASs in the surrounding environment would lead to an increase in PFASs in the human body. Drinking and dietary intake have been considered the main routes by which PFASs enter the human body [32]. Currently, short-chain PFASs, especially PFBA, have become the major PFAS contaminants in Chinese drinking water, wheat and vegetables [31, 33, 34, 35]. Evidence has also shown that ongoing exposure to even relatively low concentrations of PFASs in drinking water increases human serum levels [33]. Second, the blood-follicle transfer efficiencies for PFASs decrease with increasing chain length [34]. Although PFBA was the most abundant PFAS in FF, the present study failed to confirm any association between PFBA and ART outcomes. The possible reasons include the following: First, PFBA serum half-lives are much shorter than those of PFOA (48–96 h vs 1273 days) [36]. Second, short-chain PFBA showed less cytotoxicity, inhibition of aromatase activity and alteration of cellular lipids than long-chain homologs [37]. Since environmental long-chain PFASs have gradually decreased and been replaced by short-chain PFASs in China [31, 35], the risks posed by short-chain PFASs require further investigation.

The present study has several limitations. First, our study focused on women with POR, and it may not be possible to generalize our findings to couples from the overall population of couples attempting

conception. Second, we did not consider male partner exposure. Further studies are needed to explore whether maternal or paternal plasma PFASs had effects on IVF outcomes. The small sample size limited the interpretation of this study.

In conclusion, our study supports the association of higher FF PFAS contamination being associated with a lower chance of successful pregnancy and live birth, mainly due to a reduced fertilization rate. Additionally, this study confirmed that the levels of long-chain PFAS contamination are declining in the FF. The risks posed by short-chain PFASs require further investigation.

Declarations

Ethics approval and consent to participate

All experiments were performed in strict accordance with the Ethics Committee at The First People's Hospital of Yunnan Province, P.R. China. Informed consent was obtained from all subjects. The Institutional Committee of the First People's Hospital of Yunnan Province, P.R. China, approved the experimental protocols (registration number: 2017YY138).

Consent for publication

All coauthors have seen and approved the final version of the paper and have agreed to its submission for publication. All patients signed written informed consent forms.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Patient selection: Y. C. and L. Z.; sample collection and processing: Y. C. and L. Z.; sample measurements: M. W.; data analysis and interpretation: Y. C. and L. Z.; study design: H. S. and Z. W.; manuscript drafting: Y. C. and H. S. All authors read and approved the final manuscript.

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