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Exposure to polycyclic aromatic hydrocarbons and risk for premature ovarian failure and reproductive hormones imbalance

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a typical class of persistent organic pollutants that is ubiquitous worldwide. Previous animal studies suggested that PAHs had adverse effects on female reproduction. However, the human data regarding relationship of PAHs exposure with women reproductive health, such as ovarian dysfunction, are scarce. In this case-control study, the associations of serum levels of PAHs with the risk of premature ovarian failure (POF) and reproductive hormones in Chinese women were investigated, with recruiting 157 POF patients and 217 healthy women. The serum levels of 12 types of PAHs, as well as reproductive hormones, including follicle-stimulating hormone, luteinizing hormone and anti-mullerian hormone, were determined. In the logistic regression models, most individual PAH congeners showed significantly positive correlations with the risk of POF ($p < 0.05$), except for fluorine and pyrene. Benzo(a)pyrene (BaP), as the most carcinogenic PAH congener, was observed to be significantly positively associated with the risk of POF. After adjustment for age, body mass index, educational levels and household income, per one-unit increase in the log-transformed BaP concentration was significantly correlated with 2.191-fold increased risk of POF (OR = 2.191, 95%CI: 1.634–2.938, $p < 0.05$). To the best of our knowledge, this is the first study to report an association between internal exposure levels of PAHs and the increased risk of POF in women.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs), as a class of typical persistent organic pollutants (POPs), are ubiquitous all over the world. PAHs are primarily generated during incomplete combustion of organic materials, including man-made combustion sources (vehicles emission, cigarette smoke, waste incineration and so on) as well as natural combustion sources (volcanic activities, forest fire etc.) (Shen et al., 2011; Wolska et al., 2012). Inhalation, ingestion and dermal contact are the major exposure routes to PAHs (Abdel-Shafy and Mansour, 2016). Additionally, more attention has been paid to the presence of PAHs in indoor air and settled house dust since people spend most of life indoors (Ma and Harrad, 2015). PAHs and their metabolites were frequently detected in human serum and urine samples (Tsai et al., 2004; Guo et al., 2013; Wang et al., 2015; Alhamdow et al., 2017; Yin et al., 2017; Zhang et al., 2017). Since many PAHs have toxic, mutagenic and/or carcinogenic properties, some PAHs are listed individually on the priority chemical list of United States Environmental Protection Agency (U.S. EPA) (Keith and Telliard, 1979). Thus the adverse effects of exposure to PAHs on human health have received considerable attention.

Several environmentally epidemiological studies have shown that PAHs exposure was associated with adverse outcomes in female reproductive health (Yang et al., 2015; Yin et al., 2017). For example, serum PAHs levels were positively correlated with the risk of polycystic ovary syndrome (PCOS), an ovarian disorder caused by an imbalance of reproductive hormones in women (Yang et al., 2015). A recent study reported that higher levels of PAHs in umbilical cord serum were correlated with decreased levels of estradiol and anti-mullerian hormone (AMH) as well as increased levels of follicle-stimulating hormone (FSH), which are important reproductive hormones for ovarian functions (Yin et al., 2017).

A few animal studies also have shown that PAHs exposure could cause ovarian dysfunction. For instance, maternal exposure to PAHs during pregnancy significantly diminished murine ovarian reserve in female offspring (Jurisicova et al., 2007). Postnatal exposure to benzo(a)pyrene (BaP) led to degeneration of primordial oocytes in immature rats (Kummer et al., 2013). A recent study reported that a highly carcinogenic PAH, methylcholanthrene (MCA), induced ovarian toxicity through both genomic and epigenomic changes, which were involved in the progress of premature ovarian failure (POF) (Rhon-Calderón et al., 2018). POF, also known as primary ovarian insufficiency (POI), is a subclass of ovarian disorder that is defined by amenorrhea, low levels of estrogen and AMH, and high levels of FSH and luteinizing hormone (LH) in women before the age of 40 years. Women suffering from POF are usually infertile and experience severe menopausal symptoms. Genetic, immunologic, infectious, metabolic and iatrogenic can lead to POF (Vabre et al., 2017). However, the cause of more than 75% of the cases was undetermined (Zhang et al., 2018). Recently, environmental factors, such as some pollutants, have been thought to contribute to the etiology of POF (Vabre et al., 2017; Li et al., 2018; Zhang et al., 2018; Ye and Liu, 2019). Although these PAHs-induced ovarian phenotypes observed in female mammals are similar to the clinical symptoms of women with POF, so far it is still unknown whether this association applies to human populations.

In this case-control study, a total of 157 POF patients and 217 healthy control women were recruited. The serum concentrations of 12 types of PAHs in case and control women were determined. The correlations of PAHs exposure with the odds of POF in these women were assessed by logistic regression models. The serum levels of FSH, LH and AMH in these women were also measured and the associations of PAHs exposure with these POF-related hormones were further analyzed.

1. Materials and methods

1.1. Study population and sample collection

The subjects in the present study were participants which were described elsewhere (Cao et al., 2020; Pan et al., 2019). From January 2015 to August 2017, a total of 157 POF patients and 217 controls from Zhejiang Province were recruited by the Department of Gynecology at Women's Hospital of Zhejiang University in Hangzhou, China. This study was approved by the Ethics Committee of Women's Hospital of Zhejiang University. Cases with a confirmed diagnosis of POF were ascertained by the European Society of Human Reproduction and Embryology guidelines (Webber et al., 2016). In this study, the eligibility criteria for POF cases included: (1) under 40 years of age at the first time of diagnosis; (2) amenorrhea for at least 4 months; (3) an increased FSH level >25 IU/L on two occasions >4 weeks apart; (4) patients with known causes of POF (such as karyotypic abnormalities, ovarian surgery, and autoimmune diseases etc.) were excluded. The eligibility criteria for the controls included: (1) healthy women with regular menstrual cycles; (2) without hormonal therapy in the last six months; (3) without endocrine system diseases, such as polycystic ovary syndrome, thyroid, and hyperprolactinemia etc. In addition, the controls collected from routine physical checkups were matched with cases for age and body mass index (BMI) as close as possible. The regional distributions of participants were shown in Appendix A Table S1, with 92% of cases and 97% of controls from Zhejiang Province, and less than 10% of cases or controls from other provinces in China. Informed consent was obtained from each participant. Detailed information on women's socio-demographic characteristics, menstrual cycle, reproductive history, and lifestyle, smoking and medical histories were collected through face to face interviews. Peripheral blood of patients was collected at the time of interview with a structured questionnaire. Venous blood of control women was collected during the early follicular phase of menstrual cycle (from the 1st to 5th day of the spontaneous bleeding episode), so that the control women were at the basal levels of FSH and LH when collecting blood. The blood was centrifuged immediately at 3000 r/min for 10 min, and the serum was collected in a polypropylene tube. The samples were stored at −80 °C for further chemical analysis and hormone measurement.

1.2. Chemical analysis of serum PAHs

Serum PAHs were measured with a modified method as previously described (Yin et al., 2017). Briefly, 300 μL of serum was spiked with the pre-mixed isotopically labeled internal standard. After adding 0.5 mL of formic acid and 2.5 mL of ethanol, the serum was liquid-liquid extracted with n-hexane/dichloromethane (1:1, V/V) three times. The combined organic extract was concentrated to about 1 mL and cleaned up by a chromatographic column filled with anhydrous Na_2SO_4, pre-activated silica gel and anhydrous Na_2SO_4 from bottom to top. The elution was concentrated and reconstituted in 50 μL of *n*-nonane. The concentrated sample (2 μL) was injected into a gas chromatography-triple quadrupole mass spectrometer system (Agilent 7890B GC/7000C, Agilent Inc., USA). All the targets were analyzed by mass spectrometry electron impact ionization mode. Among the 16 U.S. EPA priority controlled PAHs, indeno (1,2,3-cd) pyrene (IcdP), dibenz (a,h) anthracene (DahA) and benzo (g,h,i) perylene (BghiP) were unable to be determined in multi-reaction monitor mode (MRM) (Neal et al., 2008; Singh et al., 2008; Qin et al., 2011; Ren et al., 2011; Al-Daghri et al., 2014). Benz(a)anthracene (BaA) was not analyzed in this study because the peak was cut off by the division of retention time. Therefore, except for BaA, IcdP, DahA and BghiP, a total of 12 types of U.S. EPA precedent-controlled PAHs were detected in the serum samples, including naphthalene (NAP), acenaphthene (ACE), acenaphthylene (ACY), fluorine (FLO), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), chrysene (CHR), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF) and benzo(a)pyrene (BaP). All the PAHs were analyzed in MRM according to previous studies (Yin et al., 2017). The limit of detection (LOD) was defined as three times the signal-to-noise, which was shown in Appendix A Table S2.

Total cholesterol (CHOL) and triglycerides (TG) were determined by Roche Cobas c701 Automatic Biochem Station (Roche Diagnostics, Germany). The serum PAHs levels were normalized by total lipids (TL), which were calculated as previously described (Covaci et al., 2006): $TL = 1.12 \times CHOL + 1.33 \times TG + 1.48$.

1.3. Assessments of reproductive hormone levels

The serum LH, FSH and AMH levels were measured by an automated Roche Modular Analytics E170 immunoassay system (Roche Diagnostics, Mannheim, Germany). The LOD of the assays were 0.10 IU/L, 0.10 IU/L and 0.001 ng/mL for LH, FSH and AMH, respectively. Inter and intra-assay coefficient of variations (CVs) for all the tested hormones was less than 10%.

1.4. Quality control and quality assurance

One standard solution of PAHs was run with each batch of the samples using the same process. A blank sample was analyzed with every set of samples to check the potential contamination in the sample analysis process. The mix standards of target compounds were spiked into the matrix and ran with the sample extraction and clean-up process ($n = 3$). The recovery ratios of 12 types of PAHs were shown in Appendix A Table S2. Isotopically labeled solutions of PAHs (1,4-Dichlorobenzene-d_4, Acenaphthene-d_{10}, Chrysene-d_{12}, Naphthalene-d_8, Perylene-d_{12}, Phenanthrene-d_{10}, bought from Accustandard Z-014J) were added to each sample as the internal standards. Recoveries of the isotope-labeled standards as above for the analysis process were in the range of 68.27%–99.13%.

1.5. Statistical analysis

SPSS 16.0 software was used for statistical analysis. The data below LOD was set to LOD/$\sqrt{2}$ (Yin et al., 2017; Li et al., 2018). The PAHs concentrations did not follow normal distribution. Therefore, the concentrations of PAHs were log-10-transformed to obtain normal distributions. The distributions of LH, FSH and AMH levels were categorized into quartiles because they were not normally distributed. Mann-Whitney U test was used to compare the differences in serum PAHs levels and reproductive hormone levels between the POF cases and control groups. Correlations among 12 PAHs were determined by Pearson correlation analysis. Risk of POF associated with PAHs levels was estimated by the odds ratio (OR) with 95% confidence interval (CI). A multinomial logistic regression model was used to estimate the associations between hormone levels and PAHs exposure. Age, body mass index (BMI), educational level and annual household income were selected as potential confounders according to previous studies (Li et al., 2018). All tests were two-sided and a level of $p < 0.05$ was used to define statistical significance.

2. Results

2.1. Characteristics of the participants and serum levels of reproductive hormone

Demographic characteristic of the POF cases and controls were presented in Table 1. The mean age of these women was (33 ± 6) years in the control group and (34 ± 6) years in cases, respectively. There were no significant differences between the cases and controls based on mean age, the distribution of BMI and parity. However, the annual household income and education levels were higher in control groups compared with the POF cases. Only 2 women in POF group reported an occasionally active smoking. The distribution of residence of the cases and controls were listed in Appendix A Table S2.

The mean levels of serum reproductive hormones in POF cases and controls were presented in Table 1. As expected, all women in POF group had FSH levels higher than 25 IU/L. Women with POF had significantly higher FSH and LH levels but lower AMH levels compared with those in the control group ($p < 0.05$). The mean levels of FSH, LH and AMH of POF cases were 67.50 IU/L, 39.76 IU/L and 0.12 ng/mL, respectively. The mean levels of FSH, LH and AMH of the control group were 6.80 IU/L, 4.78 IU/L and 3.08 ng/mL, respectively.

2.2. Serum concentrations of PAHs

The detection frequency and distribution of 12 PAHs were shown in Table 2. All the PAH were detected in at least 70% of the samples. The detection ratios of most PAHs were higher

Table 1 – Demographic characteristics of premature ovarian failure (POF) cases (N = 157) and controls (N = 217).

Characteristics	Cases		Controls	
	No.	% or (Mean ± SD)	No.	% or (Mean ± SD)
Age at Enrollment (year)	157	34 ± 6	217	33 ± 6
Age at Menopause (year)	157	33 ± 6	–	–
BMI (kg/m^2)[a]	N = 157		N = 211	
<18.5	15	9.6	24	11.4
18.5–24.0	119	75.8	156	73.9
≥24.0	23	14.6	31	11.7
Education	N = 157		N = 215	
Elementary school	46	29.7	25	11.6
High school	53	34.2	17	7.9
College	56	35.7	173	80.5
Annual Household Income (RMB yuan)	N = 154		N = 215	
<30,000	42	27.3	10	4.7
30,000-100,0000	62	40.3	56	26.0
>100,000	50	32.5	149	39.3
Active Smoking	N = 126		N = 186	
Yes	2	1.6	0	0
No	124	98.4	186	100
Parity	N = 157		N = 216	
0	15	9.6	60	27.8
1	119	75.8	137	63.4
≥2	23	14.6	19	8.8
Reproductive Hormone Levels				
FSH (IU/L)	157	67.50 ± 30.49	217	6.80 ± 2.61
LH (IU/L)	155	39.76 ± 20.04	217	4.78 ± 2.61
AMH (ng/mL)	145	0.12 ± 0.67	217	3.08 ± 2.35

-: no data is available because the women in control group are healthy with regular menstrual cycles.
FSH: follicle-stimulating hormone; LH: luteinizing hormone; AMH: anti-mullerian hormone.
[a] Grouped according to the Criteria of Weight for Adults of the People's Republic of China (WS/T 428-2013).

than 95%, except that the detection ratios of ACE and BaP were 87.26% and 84.71% in POF cases, 73.27% and 70.97% in control group, respectively. The distribution of 12 types of PAHs was similar in control and case groups. Among these 12 PAHs, NAP and PYR were the most prevalent, which contributed 59.43% (33.70% + 25.73%) of all the PAHs in the POF cases and 58.06% (31.14% + 26.92%) of all the PAHs in the control group, respectively.

Except for FLO, the mean concentrations of each PAH congener detected in this study was significantly higher in the POF cases than that in control groups ($p < 0.05$, Table 2). Although the mean concentration of FLO was higher in the control group than that in POF cases, there were no significant differences ($p = 0.329$, Table 2). The mean value of ΣPAHs (the sum of 12 PAH congener levels) in POF group was 242.77 μg/L (47.60 μg/g), which was significantly higher than that in control group (193.63 μg/L, 39.26 μg/g) ($p < 0.05$). In addition, a Pearson correlation analysis was performed to examine the association among 12 types of PAHs (Appendix A Table S3). Most of them were significantly correlated, but only NAP was not significantly associated with other types of PAHs (Appendix A Table S3).

2.3. Association of PAHs exposure with POF

Binary logistic regression models were used to determine the association between serum levels of PAHs and the risk of POF (Table 3). Since most of the PAH conger was correlated with each other, each PAH conger and the sum of 12-PAHs were added into the logistic regression models separately to reduce the effects caused by the colinear. Except for FLO and PYR, most PAH congeners and ΣPAHs showed significantly positive correlations with the risk of POF ($p < 0.05$, Table 3). BaP, as the most carcinogenic PAH congener, was observed to be significantly positively associated with the risk of POF (Table 3). In the unadjusted model, each log unit increase in serum concentration of BaP was significantly associated with 1.667-fold increased risk of POF (OR = 1.667, 95%CI: 1.318–2.110, $p < 0.05$). After adjustment for age, BMI, educational levels and household income, per one-unit increase in the log-transformed BaP concentration was significantly correlated with 2.191-fold increased risk of POF (OR = 2.191, 95%CI: 1.634–2.938, $p < 0.05$). Other PAH congeners, such as NAP, ACE, ACY, PHE, ANT, FLA, CHR, BbF and BkF, were also found to be significantly positively associated with the risk of POF in both unadjusted and adjusted models ($p < 0.05$). A significantly positive association was also found between ΣPAHs levels with the risk of POF. In adjusted models, per one-unit increase in the log-transformed ΣPAHs concentration was significantly associated with 1.879-fold increased risk of POF (OR = 1.879, 95%CI: 1.423–2.481, $p < 0.05$).

2.4. Association of PAHs exposure with serum reproductive hormone levels

The relationship of PAHs exposure with reproductive hormone levels was assessed using multivariate logistic regression models. The levels of FSH, LH and AMH were divided into

Table 2 – Distribution of serum lipid adjusted concentrations of 12-type PAHs congeners and sum of 12 PAHs in premature ovarian failure (POF) cases (N = 157) and controls (N = 217).

PAHs (μg/g lipid)	Detected (N)	Detected ratio (%)	Contribution to 12-PAHs	Mean ± SD	Percentiles[a]					p-Value[b]
					5th	25th	50th	75th	95th	
NAP										
Cases	157	100.00%	33.70%	16.04 ± 20.91	5.09	9.37	12.67	16.87	26.33	<0.001
Controls	217	100.00%	31.14%	12.23 ± 10.30	3.59	7.30	10.05	13.65	28.08	
ACE										
Cases	137	87.26%	2.71%	1.29 ± 1.23	0.07	0.68	1.03	1.48	2.95	0.002
Controls	159	73.27%	2.95%	1.16 ± 1.62	0.06	0.09	0.69	1.47	3.66	
ACY										
Cases	152	96.82%	1.81%	0.86 ± 0.51	0.13	0.42	0.84	1.16	2.03	0.004
Controls	209	96.31%	1.96%	0.77 ± 0.70	0.02	0.21	0.58	1.07	2.28	
FLO										
Cases	155	98.73%	2.66%	1.26 ± 0.88	0.39	0.83	1.10	1.40	3.31	0.329
Controls	214	98.62%	4.51%	1.77 ± 2.09	0.15	0.50	0.96	2.11	6.64	
PHE										
Cases	155	98.73%	14.04%	6.68 ± 4.47	2.10	3.86	5.64	8.30	14.28	<0.001
Controls	217	100.00%	14.38%	5.64 ± 4.99	1.54	2.71	4.10	6.67	15.38	
ANT										
Cases	155	98.73%	0.90%	0.43 ± 0.47	0.09	0.22	0.33	0.49	0.99	0.002
Controls	208	95.85%	0.98%	0.39 ± 0.47	0.01	0.14	0.25	0.46	1.13	
FLA										
Cases	155	98.73%	11.20%	5.33 ± 3.67	1.42	3.03	4.31	6.93	11.59	<0.001
Controls	217	100.00%	11.79%	4.63 ± 4.54	0.79	2.20	3.07	5.20	13.21	
PYR										
Cases	155	98.73%	25.73%	12.25 ± 10.75	2.42	6.67	10.09	14.36	29.78	0.001
Controls	217	100.00%	26.92%	10.57 ± 11.18	1.51	4.52	7.45	12.47	31.65	
CHR										
Cases	155	98.73%	3.21%	1.53 ± 1.52	0.17	0.62	1.13	1.77	4.73	<0.001
Controls	217	100.00%	2.39%	0.94 ± 1.20	0.15	0.27	0.42	1.31	3.09	
BbF										
Cases	153	97.45%	1.49%	0.71 ± 0.68	0.08	0.29	0.60	0.86	1.99	<0.001
Controls	215	99.08%	1.24%	0.49 ± 0.72	0.05	0.10	0.16	0.58	2.53	
BkF										
Cases	153	97.45%	1.40%	0.67 ± 0.70	0.05	0.22	0.46	0.86	2.12	<0.001
Controls	215	99.08%	1.03%	0.41 ± 0.72	0.04	0.07	0.13	0.53	1.42	
BaP										
Cases	133	84.71%	1.17%	0.56 ± 1.25	0.00	0.16	0.27	0.48	1.25	<0.001
Controls	154	70.97%	0.71%	0.28 ± 0.43	0.00	0.00	0.14	0.33	1.10	
ΣPAHs										
Cases				47.60 ± 29	20.76	30.69	40.63	54.29	113.53	<0.001
Controls				39.26 ± 27.15	13.28	22.49	30.97	46.60	99.22	

[a] The total lipids data of 14 POF cases and 3 control samples were missing.; [b] Mann-Whitney U test.

quarters because of the skewed distribution. As shown in Fig. 1 and Appendix A Table S4, FSH levels were significantly positively associated with the serum levels of NAP, ACE, ACY, PHE, ANT, FLA, CHR, BbF, BkF and BaP (p for trend < 0.05). LH levels were significantly positively correlated with the serum levels of NAP, ACE, ANT, CHR, BbF, BkF and BaP (p for trend < 0.05, Fig. 2 and Appendix A Table S5). There are significantly inverse associations between AMH levels and the concentrations of serum NAP, ACE, ACY, FLO, ANT, FLA, CHR, BbF, BkF and BaP (p for trend < 0.05, Fig. 3 and Appendix A Table S6). The ORs showed that a total of 7 PAHs, that is NAP, ACE, ANT, CHR, BbF, BkF and BaP, were significantly associated with these three POF-related hormones (p for trend < 0.05, Figs. 1–3, Appendix A Tables S4–S6). Moreover, the concentration of ΣPAHs was also positively associated with FSH and LH levels, whereas inversely correlated with AMH levels (Figs. 1–3, Appendix A Tables S4–S6).

Among these PAHs, BaP exhibited strongest associations with these reproductive hormones in the logistic regression models (Figs. 1–3, Appendix A Tables S4–S6). In adjusted model, compared with the lowest quartile of FSH levels, each log increase in serum concentration of BaP was significantly associated with 64.9% induction in odds of being in the third quartile (OR = 1.649, 95%CI: 1.178–2.307, p = 0.024), and 269.7% induction in odds of being in the fourth quartiles of FSH levels (OR = 2.697, 95%CI: 1.828–3.980, p < 0.05), with a significantly positive trend (p for trend < 0.001) (Fig. 1b, Appendix A Table S4). Compared with the lowest quartile of LH, per unit increase in log-transformed BaP concentration was associated with 55.1% and 92.3% induction in odds of being in the third and the highest quartiles in adjusted models, respectively (OR = 1.551, 95%CI: 1.127–2.134, p = 0.007 for the third quartile; OR = 1.923, 95%CI: 1.359–2.723, p < 0.05 for the fourth quartile) (Fig. 2b,

Table 3 – Estimated standardized coefficients (β) for premature ovarian failure (POF) associated with the serum levels of 12 types of PAH congeners in binary logistic regression models.

PAHs	Unadjusted Model			Adjusted Model[a]		
	β	OR (95%CI)	*p*-Value	β	OR (95%CI)	*p*-Value
NAP	0.421	1.524 (1.204–1.930)	<0.001	0.456	1.578 (1.198–2.078)	0.001
ACE	0.401	1.493 (1.188–1.877)	0.001	0.447	1.564 (1.201–2.037)	0.001
ACY	0.344	1.410 (1.112–1.788)	0.005	0.387	1.473 (1.104–1.965)	0.008
FLO	0.038	1.039 (0.839–1.287)	0.726	0.126	1.134 (0.881–1.460)	0.330
PHE	0.261	1.298 (1.017–1.656)	0.036	0.380	1.463 (1.095–1.954)	0.010
ANT	0.365	1.440 (1.127–1.839)	0.003	0.548	1.730 (1.273–2.351)	<0.001
FLA	0.240	1.271 (1.002–1.612)	0.048	0.324	1.382 (1.052–1.816)	0.020
PYR	0.127	1.136 (0.901–1.432)	0.282	0.152	1.164 (0.902–1.501)	0.243
CHR	0.621	1.860 (1.439–2.404)	<0.001	0.796	2.217 (1.616–3.040)	<0.001
BbF	0.646	1.907 (1.479–2.4600)	<0.001	0.745	2.107 (1.556–2.854)	<0.001
BkF	0.682	1.978 (1.524–2.568)	<0.001	0.832	2.298 (1.672–3.158)	<0.001
BaP	0.511	1.667 (1.318–2.110)	<0.001	0.784	2.191 (1.634–2.938)	<0.001
ΣPAHs	0.459	1.583 (1.258–1.993)	<0.001	0.631	1.879 (1.423–2.481)	<0.001

[a] The adjusted model included age, BMI, annual household income and education.

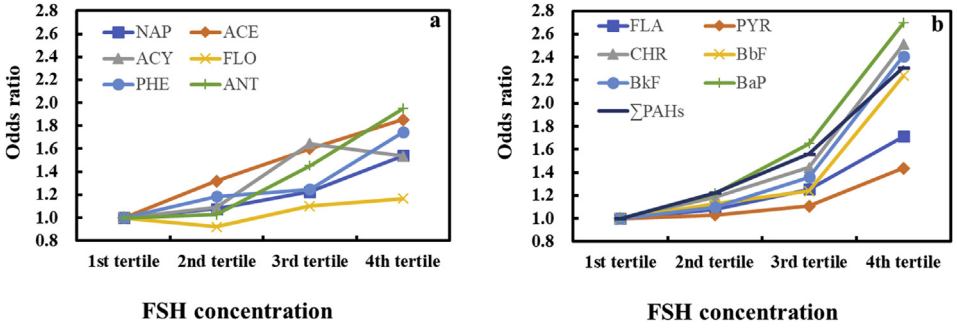

Fig. 1 – Odds ratio for serum levels of follicle-stimulating hormone (FSH) associated with PAH congeners (a) NAP, ACE, ACY, FLO PHE, ANT, (b) FLA, PYR, CHR, BbF, BkF, BaP, ΣPAHs in adjusted logistic regression models.

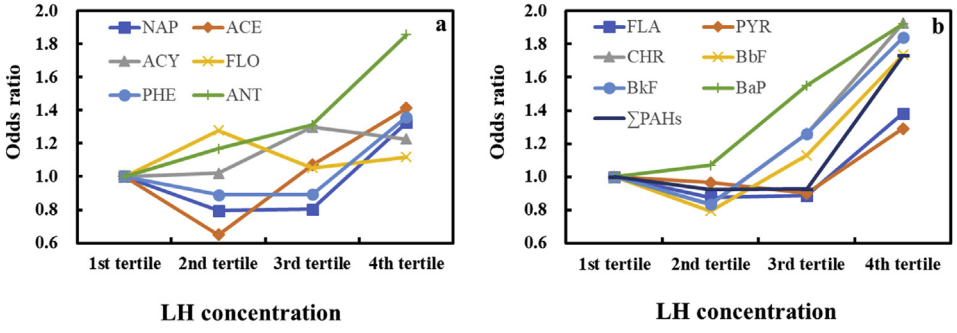

Fig. 2 – Odds ratio for serum levels of luteinizing hormone (LH) associated with PAH congeners (a) NAP, ACE, ACY, FLO, PHE, ANT, (b) FLA, PYR, CHR, BbF, BkF, BaP, ΣPAHs in adjusted logistic regression models.

Appendix A Table S5). Compared with being in the first quartile of AMH, adjusting models for potential confounders resulted in 49.3%, 60.3% and 63.9% reduction in odds of being in the second, third and fourth quartiles of AMH levels per log increase in BaP concentration (OR = 0.507, 95%CI: 0.345–0.746, $p = 0.001$ for the second quartile; OR = 0.397, 95% CI: 0.272–0.578, $p < 0.05$ for the third quartile; OR = 0.361, 95% CI: 0.248–0.527, $p < 0.05$ for the fourth quartile) (p for trend < 0.001) (Fig. 3b, Appendix A Table S6).

3. Discussion

In the present study, the detection ratios for 12 individual PAH congeners ranged from 70.97% to 100%. The mean levels of Σ PAHs in POF cases and control were 242.77 μg/L and 193.63 μg/L (47.60 and 39.26 μg/g lipid), respectively. Since PAHs are ubiquitous worldwide, many biomonitoring or epidemiological studies have measured the serum levels of PAHs in

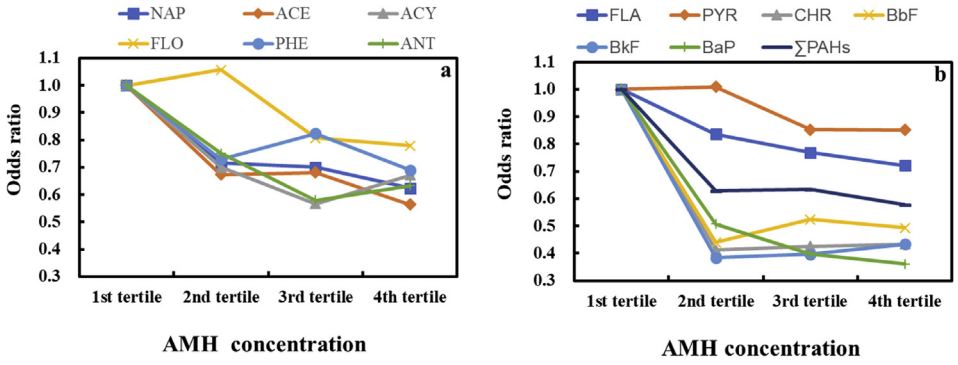

Fig. 3 – Odds ratio for serum levels of anti-mullerian hormone (AMH)associated with PAH congeners (a) NAP, ACE, ACY, FLO, PHE, ANT, (b) FLA, PYR, CHR, BbF, BkF, BaP, ΣPAHs in adjusted logistic regression models.

women populations (Song et al., 2013; Wang et al., 2015; Zhang et al., 2017). For example, the mean serum concentration of 13 PAHs (ANY, ACE, ACY, FLO, PHE, ANT, FLA, PYR, CHR, BbF, BaP, DahA, and BghiP) were up to 1120 μg/L in the women with age range 18–40 years from one rural area of Egypt, which was much higher than those in this study (Loutfy et al., 2017). The serum mean levels of total 9 PAHs (including ANT, PHE, FLA, NAP, ACY, PYR, BaP, BkF and DahA) in Indian women with benign and malignant breast lesions or healthy women were 185.99 and 200.74 μg/L or 142.05 μg/L, respectively, which were comparable to the mean levels in the population of this study (Singh et al., 2008). The median serum level of BaP reported in Chinese women in Shanxi Province (0.214 μg/g) was comparable to that in the POF women (0.27 μg/g) in this study (Wang et al., 2015). The differentially internal exposure levels of PAHs in women from different regions may depend on the various local pollution characteristics.

Smoking has been identified as one of the most important predictors of internal exposure levels of PAHs (Li et al., 2008). A few epidemiological studies have shown that cigarette smoking was significantly correlated with POF and earlier menopause (Harlow and Signorello, 2000; Prospero et al., 2003; Chang et al., 2007). However, since there were only 2 active smoking participants in the case group in this study, there was no correlation between smoking and the risk of POF or PAHs concentrations. Therefore, active smoking was not the main exposure pathway of PAHs in women population in this study.

These 12 types of PAH congeners measured in this study could be grouped by lower-molecular-weight PAHs (L-PAHs) (including NAP, ACE, ACY, FLO, PHE, ANT, FLA, and PYR) and high-molecular-weight PAHs (H-PAHs) (including CHR, BbF, BkF and BaP) (Yu et al., 2015; Yin et al., 2017). Noticeably, H-PAHs are associated with higher risks of occurrence of POF and imbalance of ovarian function related hormones than those of L-PAHs in both unadjusted and adjusted models. Indeed, H-PAHs show greater toxicity and carcinogenicity than L-PAHs in toxicology testing. For instance, the chronic oral reference dose (RfD, mg/(kg·day)) of BaP is 3×10^{-4}, which is much lower than that of NAP, ACE, FLO, ANT, FLA and PYR (2×10^{-2}, 6×10^{-2}, 4×10^{-2}, 3×10^{-1}, 4×10^{-2} and 3×10^{-2}, respectively) (U.S. EPA). Moreover, BaP was considered carcinogenic to human by U.S. EPA, while most of L-PAHs, including ACY, FLO, PHE, ANT, FLA and PYR, were not classifiable to human carcinogenicity (U.S. EPA). Our findings suggest that the exposure of PAH congeners with higher toxicity and carcinogenicity is associated with the higher risk of ovarian dysfunction in women.

To the best of our knowledge, this is the first study that revealed an association between internal exposure levels of PAHs and the increased risk of POF in women. Although no similar environmentally epidemiological studies can be compared with our results, a few animal studies in agreement with our observations reported that PAHs exposure could induce POF-like phenotypes (Jurisicova et al., 2007; Lim et al., 2013; Matikainen et al., 2001; Sadeu and Foster, 2011, 2013). For instance, prenatal exposure to BaP caused decreased fertility, ovarian follicle depletion and ovarian tumorigenesis in female mice (Lim et al., 2013). Perinatal exposure to the mixture of 2 prototypical PAHs, 7,12-dimethylbenz(a)anthracene and BaP, resulted in accelerated loss of primordial follicles, leading to premature exhaustion of the ovarian pool that is the main phenotype of POF (Jurisicova et al., 2007). It was well documented that most PAHs with coplanar structure act as ligands for the aryl hydrocarbon receptor (AhR) to exert their toxic effects (Matikainen et al., 2001; Ji et al., 2019). An *in vitro* study reported that BaP delayed ovarian follicular development and decreased follicle viability through activation of AhR signaling (Sadeu and Foster, 2013). A recent study showed that exposure to low doses of MCA altered the expression of a subset of ovarian genes involved in POF and most of these changes occurred in an AhR-dependent mechanism (Shen et al., 2011). Therefore, it is possible that the increased risk of POF in females by PAHs exposure observed in this study is attributed to PAHs-induced deprivation of ovarian primordial follicle pool via activation of AhR signaling.

FSH and LH are gonadotropin hormones secreted by the pituitary gonadotropes and play important roles in the regulation of ovarian and follicular development in females. In this study, higher PAHs exposure was found to be correlated with significantly higher levels of FSH and LH. In accordance with our study, the relationship between PAHs exposure with FSH/LH levels has been reported in a few studies (Luderer et al., 2017; Yin et al., 2017). Exposure to PAHs was positively associated with FSH levels in umbilical cord serum (Yin et al., 2017). Positive associations between urinary concentrations of hydroxylated PAH metabolites with follicular phase LH levels were observed in women (Luderer et al., 2017). Since elevated FSH and LH are one of the main clinical symptoms of

POF, the positive association of PAHs exposure with FSH and LH levels in this study further supported the relationship of PAHs exposure with the increased risk of POF.

This study also showed that higher PAHs exposure was significantly correlated with lower levels of AMH. Only one previous study has shown that exposure to PAHs was inversely associated with AMH concentrations in umbilical cord serum (Yin et al., 2017), which was in consistent with the inverse correlation of PAHs with AMH in this study. AMH is secreted by the ovarian granulosa cells and plays a crucial role in folliculogenesis (Hayes et al., 2016). AMH level is capable to determine the size of the ovarian follicular reserve, thus decreased AMH is used as a clinical marker in infertility and ovarian disorders such as POF and PCOS (Pigny et al., 2003; Visser et al., 2012; Hayes et al., 2016; Convissar et al., 2017). The inverse relationship of PAHs exposure with AMH levels also provided further support to the findings of a positive association between PAHs and the risk of POF.

This study has strength, such as the relatively larger number of subjects of POF patients and the accurate exposure determination of PAHs using internal exposure biomarkers. Nonetheless, some limitations cannot be neglected. First, only 12 types of PAHs were accurately measured in this study, which may be unable to reflect all the PAHs exposure in these women. Moreover, the information about passive smoking was not included in the questionnaire. In fact, both active smoking and passive smoking were sources of PAHs exposure, so controlling for them in the logistic models may result in over-adjustment. Third, the annual household income and educational levels were higher in the control group than those in the case group, which might result in potential bias. Additionally, it was a limitation that we did not investigate the effects of other individual's lifestyle on POF, such as dietary nutrition and excessive weight loss, which would result in insufficient estrogen synthesis. Finally, the distribution of residence of POF patients and healthy control women were not uniform, which does make it difficult to completely eliminate the impact of different distributions of different PAHs (or other pollutants) in different cities on the analysis.

4. Conclusions

Our case-control study revealed that exposure to PAHs was associated with an increased risk of POF. Furthermore, serum PAHs levels were significantly associated with higher concentrations of FSH and LH but lower concentrations of AMH. This study suggested that PAHs exposure in daily life posed a risk to female reproductive health and highlighted the urgent need to reduce PAHs exposure for women at childbearing age. Further studies are needed for more evidence of these relationships in large-scale population as well as the underlying mechanisms between PAHs exposure and POF.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2019.12.015.

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