SUPPLEMENTARY MATERIALS

Materials: Section S1
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Section S1: Study design

1 Inclusion and Exclusion Criteria

1.1 PCOS diagnostic criteria

Rotterdam criteria was used as the diagnostic criteria for PCOS[1]. At least two of the following three criteria should be met: 1) Oligo- or anovulation; 2) Clinical and/or biochemical signs of hyperandrogenism; 3) Polycystic ovaries after exclusion of other etiologies.

1.2 Inclusion criteria

Women aged 25–37 years with a body mass index of 18.5–28 kg/m^2 were included.

1.3 Exclusion criteria

Participants with endometriosis, adenomyosis, hyperprolactinemia, thyroid disorders, hypertension, diabetes mellitus, hyperlipidemia, hyperprogesteronemia, congenital adrenal hyperplasia or androgen-secreting tumors were excluded.

2 Study Population

During 2018–2021, women who had undergone *in vitro* fertilization–embryo transfer or intracytoplasmic sperm injection–embryo transfer treatments at Yantai Yuhuangding Hospital were recruited. A total of 5,889 women with follicular fluid samples were initially included. According to the inclusion and exclusion criteria mentioned, 200 women with PCOS were included (noted as cases). To enlarge the controls, 900 women meeting the inclusion and exclusion criteria but without PCOS were randomly selected with loose matching conditions, resulting in the ratio of control/case = 4.5. Among them, four participants of controls provided insufficient follicular fluid samples for analyses. Thus, 200 cases and 896 controls (1,196 in total) were used for analyses. The informed consent was finished by all participants, and ethical regulations regarding human subjects were all complied with. Approval was obtained from the Institutional Review Board of Yuhuangding Hospital of Yantai for this study.

3 Calculation of Sample Size

The sample size was calculated based on previous studies. A case-control study including 369 PCOS cases and 441 controls in Anhui Province indicated that higher likelihoods of PCOS were observed in the high exposure groups of Pb, As, and Ba with the adjusted odds ratios of 2.08 (third *vs.* first tertile, 95% *CI*: 1.42–3.04), 2.83 (third *vs.* first tertile, 95% *CI*: 1.93–4.15), and 1.89 (third *vs.* first tertile, 95% *CI*: 1.32–2.72), respectively[2]. Based on the above information, the sample size for the cases (*N1*) and controls (*N2*) were calculated using PASS 2021 by assuming the conditions of odds ratio = 2.0, α = 0.05, β = 0.20, control group proportion = 0.5, *N2*/*N1* = 1, resulting in the sample size of 137 for each group. Thus, it is considered that sample size in this study had stronger statistical power to investigate associations between metal(loid) exposure and PCOS risk in women of childbearing age.

Supplementary Table S1. Spearman correlations among twenty-nine metal(loid)s analyzed in follicular fluid

r^a	P^a																											
	Al	Ge	Ti	Cr	Mn	As	Se	Sr	Li	Co	Ni	Mo	Ag	Cd	Sn	Sb	Ba	Cs	U	Cu	Zn	Rb	Hg	Pb	La	Ce	Pr	Nd	Y
Al		0.000	0.000	0.004	0.000	0.000	0.005	0.034	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.426	0.000	0.001	0.000	0.486	0.136	0.000	0.000	0.000	0.000	0.000	0.000
Ge	0.19		0.000	0.005	0.139	0.000	0.000	0.312	0.994	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.326	0.000	0.073	0.000	0.000	0.000	0.700	0.029	0.000	0.000	0.000	0.000	0.000
Ti	0.16	−0.32		0.002	0.000	0.225	0.002	0.494	0.654	0.000	0.000	0.000	0.002	0.000	0.694	0.047	0.527	0.000	0.050	0.013	0.048	0.005	0.539	0.000	0.000	0.000	0.007	0.106	0.004
Cr	0.09	−0.09	0.10		0.000	0.428	0.107	0.066	0.006	0.000	0.000	0.000	0.958	0.958	0.007	0.001	0.003	0.213	0.000	0.001	0.345	0.033	0.136	0.000	0.000	0.003	0.001	0.342	0.656
Mn	0.36	−0.04	0.18	0.43		0.456	0.000	0.012	0.028	0.069	0.000	0.013	0.108	0.085	0.042	0.000	0.000	0.078	0.000	0.000	0.927	0.001	0.879	0.000	0.000	0.000	0.000	0.000	0.176
As	0.17	0.11	0.04	0.02	0.02		0.000	0.000	0.001	0.028	0.173	0.006	0.000	0.000	0.018	0.029	0.000	0.000	0.352	0.001	0.086	0.000	0.000	0.002	0.014	0.000	0.001	0.031	0.000
Se	0.09	0.29	0.09	−0.05	−0.14	0.29		0.708	0.951	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.794	0.001	0.074	0.000	0.000	0.000
Sr	0.06	−0.03	−0.02	0.06	0.08	0.15	−0.01		0.111	0.185	0.041	0.703	0.031	0.839	0.925	0.877	0.199	0.007	0.000	0.740	0.246	0.001	0.415	0.006	0.014	0.006	0.030	0.712	0.553
Li	0.10	0.00	−0.01	0.08	0.07	0.10	0.00	0.05		0.003	0.007	0.002	0.944	0.100	0.082	0.018	0.004	0.117	0.010	0.111	0.612	0.529	0.003	0.027	0.625	0.012	0.345	0.038	0.970
Co	0.18	0.47	−0.22	0.11	0.05	0.07	0.16	0.04	0.09		0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.424	0.000	0.000	0.000	0.005	0.216	0.000	0.000	0.000	0.000	0.000
Ni	0.22	0.40	−0.22	0.23	0.31	0.04	0.07	0.06	0.08	0.58		0.000	0.000	0.000	0.000	0.000	0.000	0.172	0.000	0.000	0.000	0.008	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Mo	0.17	0.48	−0.13	0.31	0.08	0.08	0.25	−0.01	0.09	0.48	0.41		0.000	0.000	0.000	0.000	0.292	0.000	0.117	0.000	0.000	0.000	0.330	0.000	0.000	0.000	0.000	0.000	0.000
Ag	0.28	0.53	−0.09	0.00	−0.05	0.19	0.45	0.06	0.00	0.36	0.28	0.42		0.000	0.000	0.000	0.596	0.000	0.874	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000
Cd	0.25	0.62	−0.18	0.00	−0.05	0.12	0.35	−0.01	0.05	0.49	0.39	0.51	0.55		0.000	0.000	0.231	0.000	0.640	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000
Sn	0.35	0.25	−0.01	−0.08	0.06	0.07	0.18	0.00	0.05	0.23	0.19	0.14	0.28	0.30		0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Sb	0.32	0.42	−0.06	0.10	0.15	0.07	0.24	0.00	0.07	0.33	0.35	0.48	0.45	0.48	0.27		0.004	0.000	0.007	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Ba	0.49	0.03	−0.02	0.09	0.27	0.15	−0.07	0.04	0.09	0.09	0.17	0.03	0.02	0.04	0.11	0.09		0.050	0.000	0.023	0.008	0.039	0.408	0.000	0.000	0.000	0.000	0.000	0.037
Cs	0.02	0.12	0.11	−0.04	−0.05	0.23	0.37	−0.08	0.05	0.09	0.04	0.12	0.20	0.15	0.09	0.13	−0.06		0.001	0.000	0.000	0.000	0.000	0.212	0.023	0.729	0.000	0.000	0.000
U	0.31	−0.05	0.06	0.19	0.34	0.03	−0.12	0.11	0.08	−0.02	0.19	−0.05	0.00	−0.01	0.12	0.08	0.22	−0.10		0.031	0.942	0.000	0.535	0.000	0.000	0.000	0.001	0.002	0.517
Cu	0.10	0.35	−0.08	−0.10	−0.12	0.10	0.41	−0.01	−0.05	0.23	0.19	0.28	0.44	0.31	0.15	0.21	−0.07	0.18	−0.07		0.000	0.000	0.002	0.007	0.031	0.028	0.000	0.000	0.000
Zn	0.20	0.37	0.06	−0.03	0.00	0.05	0.37	0.04	−0.02	0.22	0.17	0.25	0.38	0.31	0.18	0.26	0.08	0.19	0.00	0.34		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Rb	0.02	0.30	0.08	−0.06	−0.10	0.22	0.43	−0.10	−0.02	0.13	0.08	0.22	0.26	0.26	0.11	0.17	−0.06	0.65	−0.16	0.28	0.33		0.000	0.024	0.046	0.961	0.000	0.000	0.000
Hg	0.05	0.01	0.02	−0.05	0.00	0.13	0.30	−0.02	0.09	−0.08	−0.10	−0.03	0.13	0.07	0.10	0.08	−0.02	0.16	0.02	0.09	0.13	0.12		0.745	0.814	0.642	0.087	0.075	0.245
Pb	0.44	0.07	0.23	0.21	0.36	0.09	0.01	0.08	0.07	0.04	0.11	0.12	0.10	0.15	0.20	0.23	0.21	0.04	0.26	−0.08	0.16	0.07	−0.01		0.000	0.000	0.000	0.000	0.000
La	0.55	0.17	0.22	0.15	0.30	0.07	0.10	0.07	0.01	0.16	0.22	0.25	0.28	0.28	0.22	0.32	0.24	0.07	0.21	0.07	0.20	0.06	0.01	0.49		0.000	0.000	0.000	0.000
Ce	0.75	0.12	0.14	0.09	0.37	0.20	0.05	0.08	0.08	0.14	0.22	0.15	0.20	0.17	0.25	0.25	0.60	−0.01	0.28	0.07	0.15	0.00	−0.01	0.41	0.60		0.000	0.000	0.000
Pr	0.41	0.32	0.08	0.10	0.18	0.10	0.19	0.07	0.03	0.24	0.25	0.31	0.37	0.37	0.25	0.36	0.14	0.12	0.10	0.16	0.23	0.14	0.05	0.34	0.62	0.43		0.000	0.000
Nd	0.37	0.30	0.05	0.03	0.15	0.07	0.20	0.01	0.06	0.21	0.22	0.28	0.35	0.34	0.26	0.36	0.11	0.13	0.09	0.19	0.24	0.13	0.05	0.27	0.50	0.36	0.50		0.000
Y	0.32	0.67	−0.09	0.01	−0.04	0.14	0.42	−0.02	0.00	0.50	0.40	0.58	0.65	0.70	0.32	0.57	0.06	0.18	−0.02	0.39	0.38	0.29	0.04	0.19	0.38	0.28	0.46	0.45	

Note. [a] *r*, correlation coefficients, lower left corner; *P*, p-values, upper right corner. Al, aluminum; Ge, germanium; Ti, titanium; Cr, chromium; Mn, manganese; As, arsenic; Se, selenium; Sr, strontium; Li, lithium; Co, cobalt; Ni, nickel; Mo, molybdenum; Ag, silver; Cd, cadmium; Sn, tin; Sb, antimony; Ba, barium; Cs, cesium; U, uranium; Cu, copper; Zn, zinc; Rb, rubidium; Hg, mercury; Pb, lead; La, lanthanum; Ce, cerium; Pr, praseodymium; Nd, neodymium; Y, yttrium.

Supplementary Table S2. Spearman correlations between copper levels in follicular fluid and clinical indicators

Clinical indicators	*r*	*P*	*N*
AMH	0.211	< 0.001	1,091
LH	0.119	< 0.001	1,096
FSH	−0.133	< 0.001	1,096
T	0.131	< 0.001	1,093
FT3	0.063	0.038	1,095

Note. AMH, anti-Müllerian hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; T, testosterone; FT3, free triiodothyronine; *r*, correlation coefficient; *P*, *P*-value; *N*, sample size.

Supplementary Table S3. Mediation effect of AMH in the relationship between Cu exposure and PCOS risk

Covariates	Parameters	Estimate (95% *CI*)	*P*
Age, BMI	ACME (average)	0.04 (0.03, 0.06)	< 0.001
	ADE (average)	0.05 (0.02, 0.08)	< 0.001
	Prop. Mediated (average)	0.44 (0.30, 0.65)	< 0.001
Age, BMI, Zn	ACME (average)	0.04 (0.03, 0.06)	< 0.001
	ADE (average)	0.05 (0.02, 0.08)	< 0.001
	Prop. Mediated (average)	0.45 (0.31, 0.69)	< 0.001

Note. AMH, anti-Müllerian hormone; Cu, copper; PCOS, polycystic ovary syndrome; BMI, body mass index; Zn, zinc; ACME, average causal mediation effects; ADE, average direct effects; Prop. Mediated, the proportion of the effect of Cu on PCOS risk that goes through the mediator AMH; *CI*, confidence interval.

Supplementary Table S4. Metal(loid) levels in follicular fluid or serum of women from other populations

References	Regions	Media[a]	Groups[b]	Metal(loid)s[c], ng/mL											
				Cu	Cr	Se	Zn	Mn	Pb	Hg	As	Ti	Li	Sr	Mo
(Sun et al., 2019)[3]	Fujian China	FF	Control	920.91 ± 305.89	124.09 ± 86.76	85.98 ± 22.34	479.69 ± 218.92				37.64 ± 9.39	200.07 ± 84.85	2.78 ± 0.67	32.15 ± 13.05	1.10 ± 1.05
			PCOS	1,064.53 ± 243.75	133.37 ± 41.90	91.27 ± 20.75	527.77 ± 236.70				35.98 ± 9.36	164.83 ± 49.77	2.75 ± 0.52	34.01 ± 12.38	1.71 ± 3.13
			FTO	877.98 ± 239.73	97.75 ± 53.09	80.13 ± 19.70	506.83 ± 278.30				33.72 ± 9.50	216.99 ± 101.99	2.24 ± 0.56	28.05 ± 9.76	1.22 ± 1.94
(Schmalbrock et al., 2021)[4]	Dobl Austria	FF	1 Subfertile	555 ± 191		23 ± 10	474 ± 118								
			1 PCOS	518 ± 267		19 ± 11	402 ± 164								
			2 Subfertile	683 ± 289		31 ± 26	569 ± 144								
			2 PCOS	682 ± 381		24 ± 11	572 ± 231								
			3 Subfertile	516 ± 185		24 ± 14	571 ± 111								
			3 PCOS	479 ± 179		22 ± 5	419 ± 142								
			4 Subfertile	619 ± 176		26 ± 11	536 ± 170								
			4 PCOS	652 ± 276		24 ± 11	467 ± 174								
			5 Subfertile	507 ± 190		22 ± 11	503 ± 167								
			5 PCOS	742 ± 318		27 ± 12	591 ± 220								
(Li et al., 2017)[5]	Guangxi China	Serum	Control	1,152 (992–1,400)											
			PCOS	1,273 (1,078–1,536)											
(Zhang et al., 2022)[6]	Beijing China	Serum	Control	1,320 ± 40	1.19 (0.708–2.07)	80.06 ± 2.52	860 (750–1,010)	3.45 (2.37–5.64)	2.49 (1.30–6.20)	0.104 (0.104–0.104)					
			PCOS	1,480 ± 55	2.58 (1.01–9.31)	77.31 ± 2.01	805 (665–1,120)	3.31 (2.10–7.82)	2.31 (1.14–8.47)	0.350 (0.104–0.633)					
(Kanafchian et al., 2020)[7]	Babol Iran	Serum	Control	1,870 (1,540–2,140)											
			PCOS	2,060 (1,790–2,480)											

Note. Data was presented as mean ± standard deviation or median (interquartile range). [a] FF, follicular fluid. [b] PCOS, polycystic ovary syndrome; FTO, fallopian tube obstruction. [c] Cu, copper; Cr, chromium; Se, selenium; Zn, zinc; Mn, manganese; Pb, lead; Hg, mercury; As, arsenic; Ti, titanium; Li, lithium; Sr, strontium; Mo, molybdenum.

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