Supporting Information for

Parental HFPO-TA exposure induces intergenerational reproductive toxicity and DNA methylation alterations in zebrafish

Shasha Dong [1 #], Xiaohui Zhao [1 #], Xuan Ren [1], Jing Zhang (🖂)[2], Zhibo Liu [2], Dan Yang [1], Ning Ji [1], Yawei Shi [1], Ya Sun [1], Guanghui Ding (🖂)[1]

1 College of Environmental Science and Engineering, Dalian Maritime University, Dalian 116026, China

2 College of Environment and Chemical Engineering, Dalian University, Dalian 116622, China

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🖂 Corresponding authors

E-mail: zhangjing@dlu.edu.cn (J. Zhang); guanghuiding@dlmu.edu.cn (G. Ding)

[#] Shasha Dong and Xiaohui Zhao contributed equally.

Text S1 Total RNA extraction, reverse transcription, and qPCR

Total RNA was isolated with a Trizol reagent (TIANGEN Biotech, Beijing, China) and qualified by 1% agarose gel electrophoresis. The concentrations of RNA were determined by using a Synergy HTX multi-mode microplate reader (BioTek, USA) with Gen5 software. Then, the RNA was reverse transcribed to cDNA according to the instructions of the reverse-transcription reagent kit (Takara, Tokyo, Japan). The qPCR was carried out on a LightCycler 480 (Roche, Basel, Switzerland) using 2 × SYBR Green qPCR Master Mix (Servicebio, Wuhan, China). The gene expression was quantified using the $2^{-\Delta\Delta Ct}$ method with β-actin as the internal reference gene and ddH_2O as the negative control.

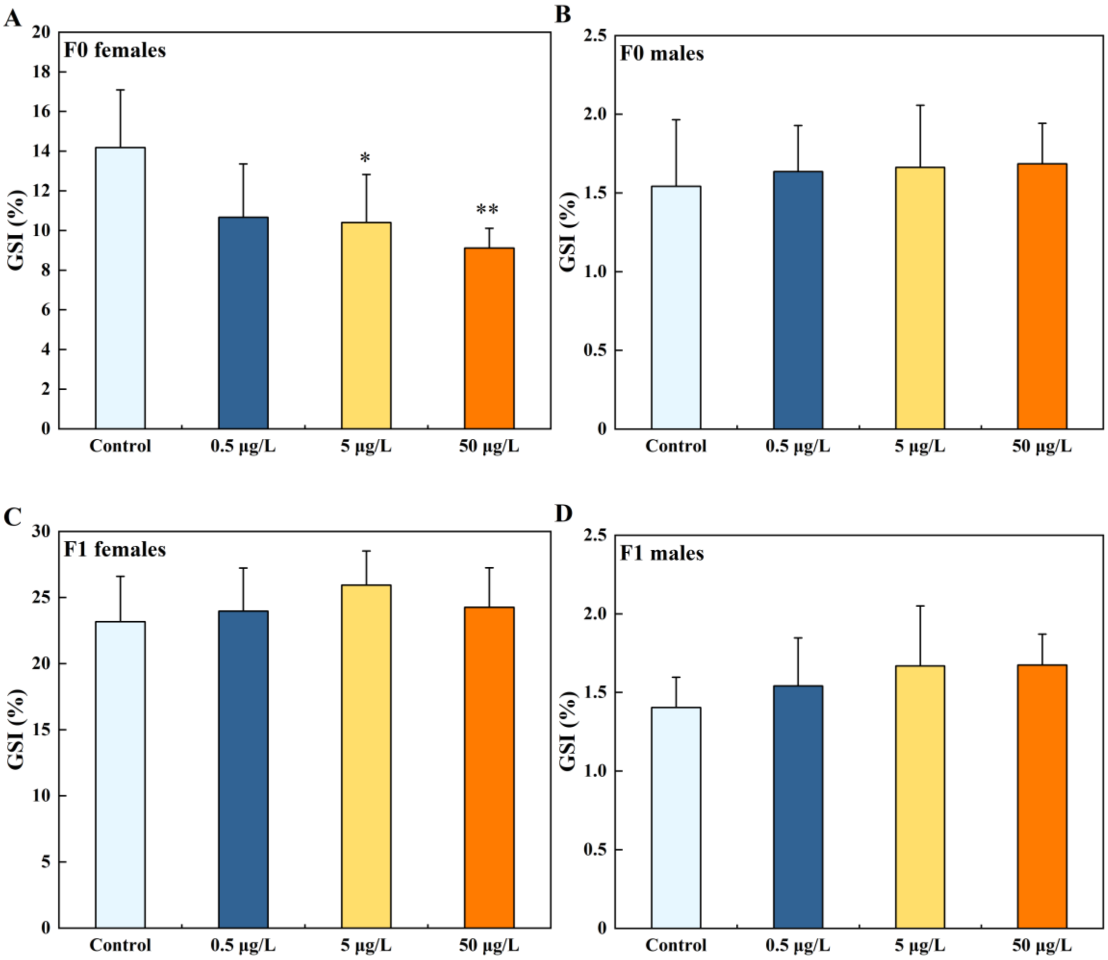


Fig. S1 Effects HFPO-TA on the gonadosomatic index (GSI) of F0 (A and B) and F1 (C and D) zebrafish. Data were expressed as mean ± SD ($n = 10$). * for $p < 0.05$ and ** for $p < 0.01$, respectively.

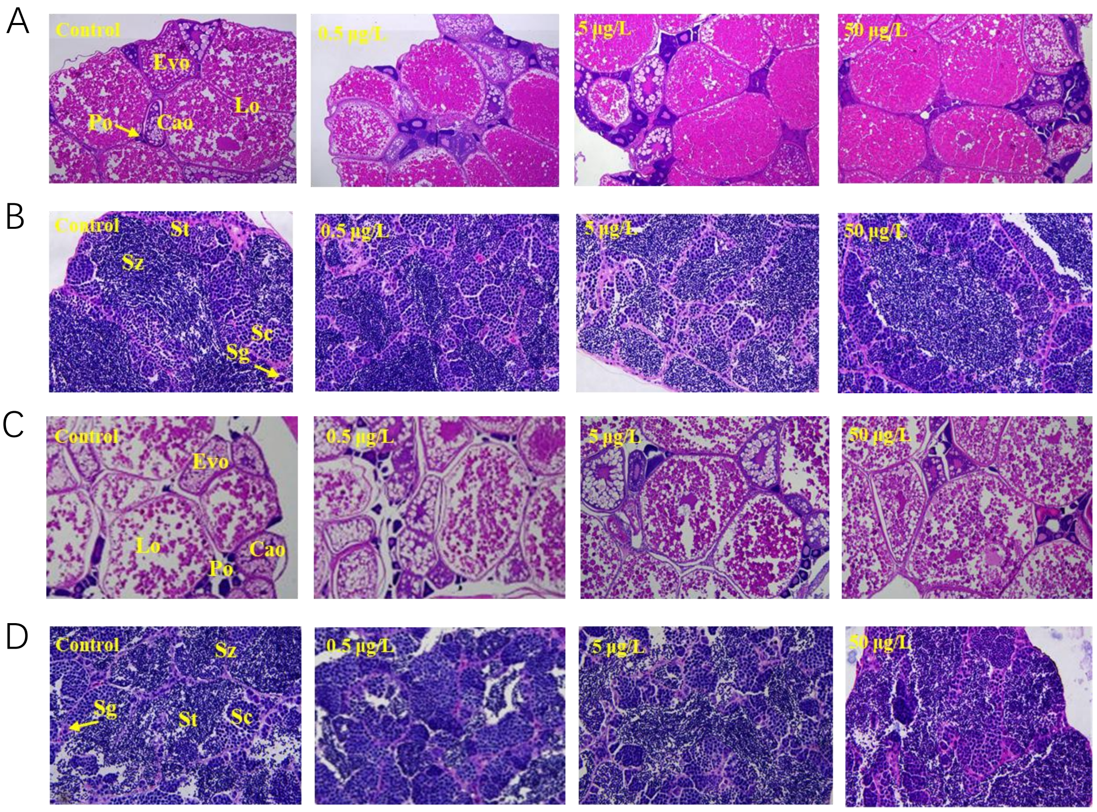
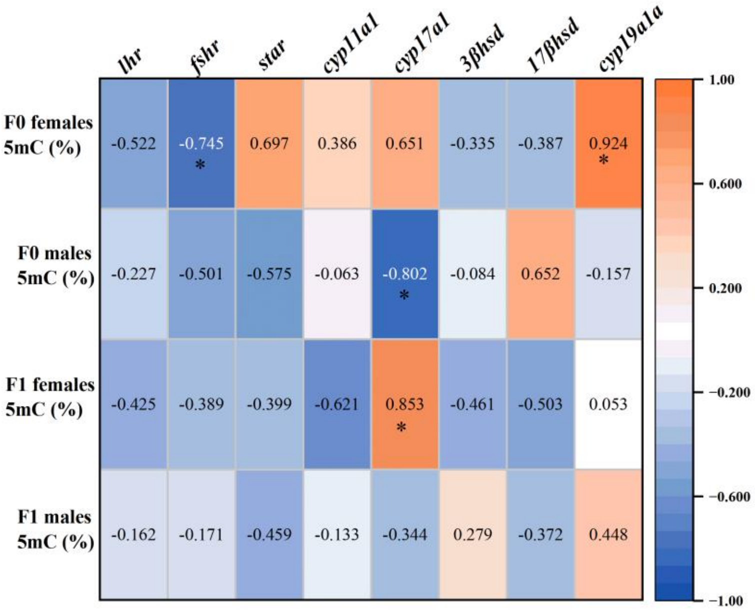


Fig. S2 Representative micrographs of F0 ovaries (A), F0 testes (B), F1 ovaries (C), and F1 testes (D).



	lhr	*fshr*	*star*	*cyp11a1*	*cyp17a1*	*3βhsd*	*17βhsd*	*cyp19a1a*
F0 females 5mC (%)	-0.522	-0.745 *	0.697	0.386	0.651	-0.335	-0.387	0.924 *
F0 males 5mC (%)	-0.227	-0.501	-0.575	-0.063	-0.802 *	-0.084	0.652	-0.157
F1 females 5mC (%)	-0.425	-0.389	-0.399	-0.621	0.853 *	-0.461	-0.503	0.053
F1 males 5mC (%)	-0.162	-0.171	-0.459	-0.133	-0.344	0.279	-0.372	0.448

Fig. S3 Correlation analyses between global DNA methylation levels and transcriptional levels of steroidogenic genes in F0 and F1 gonads. The value represents the correlation coefficient, and the asterisk indicates a significant difference ($p < 0.05$).

Table S1 Primers of target genes used in this study.

Genes name	Sequence (5'-3')	Accession No.
gnrh2	F: ACTGGTCTCACGGCTGGTATCCT R: AATCACGAATGAGGGCATCCAG	NM_181439.4
gnrh3	F: TGGTCCAGTTGTTGCTGTTAGTT R: CCTGAATGTTGCCTCCATTTC	NM_182887.2
gnrhr2	F: TCAACAAGGCGATGAGAAGAAAT R: GTAAAATGTCTCTTGCCAGCGA	NM_001144979.1
gnrhr3	F: AACAGACATGATCCCGAAGG R: AGGTTCCCGAACACAAACAG	NM_001177450.1
lhβ	F: ATGTTATTGGCTGGAAATGGTGTC R: ACGGGCTCTTGTAAACGGGAT	NM_205622.2
fshβ	F: ACAGCACACCCAGAAGGTCT R: AGCTCCCCAGTCTGTTGTGT	NM_205624.1
cyp19a1b	F: CAGCAACATCGCAAAGTGGA R: GTCCTGAGGCGTCTGTGAAC	XM_068217412.1
lhr	F: AAAAGGACGAGTCGCTGAAA R: AAAACAAGAAGCAGGGCAGA	AY424302.1
fshr	F: GCTGGCTTTTCTCCCTTCTCGC R: GCGGGATTACGGACGGTCAAGT	NM_001001812.1
star	F: GCTGAGGAGACATACAGTGAAG R: CCATCCTGGTCCTCAAGAATAC	NM_131663.1
cyp11a1	F: TCTGGATCAGTCCCTGTCTT R: CATCGTCCAGGGATCTTATTG	NM_152953.2
cyp17a1	F: GGACTCCAGTGTTGGTGAATAC R: CAGGGTTCTTCCATTCCTTCTC	NM_212806.3
3βhsd	F: GCCCATCTTTCTGCTGTATCT R: CATGGTCACTAGCTGCCTATTT	NM_212797.1
17βhsd	F: CGAAGCTCTTCTGTCCTCTTC R: TCTGCATACGCTCTTCCTATTC	AY306005.1
cyp19a1a	F: GGCTGTGGTGGATCTTCTTATC R: CAGAGTAGCAGCAGCAGTATT	NM_131154.3
erα	F: GGCTGGAGGTGTTGATGATT R: CGCATTCTCCTTCACTCCTATC	NM_152959.1

erβ	F: CTCTCAGCACCTCTTTCCTTC	AJ414566.1
	R: CCGCCTCTAGAATACAGCTAAC	
vtg1	F: CCTCTGTTATGCTGTCCCATAC	NM_001044897.3
	R: GGACTGAGTGGTGTCCAATTAT	
foxl2b	F: GCGGATTGACATTTGAACCTC	NM_001317761.1
	R: CTGCTAATTTGCGTGTAGTGC	
sox9b	F: TGGAGACTGCTGAACGAGGG	NM_131644.1
	R: CCGTCTGGGCTGGTATTTGTAG	
sox9a	F: AGTACCCGCACCTCCACAAC	NM_131643.1
	R: TTCTTCACCGACTTCCTCCG	
amh	F: CCACACGTTTCCTGATTGTCAC	NM_001007779.1
	R: TACTGCTGCCATCCACCTTTC	
dnmt1	F: GAGGAGGATGTGTTGCCAGTTA	NM_131189.2
	R: CCTCATTTTCCACGCACTTT	
dnmt3a	F: GCAAAGACCAGCACTACCCT	XM_068215441.1
	R: TTCAGAGGCCACAAGCAACA	
dnmt3b	F: GAGGCAGAGAAGAAGGCGAA	XM_009293199.4
	R: GAGTCCGCTCGTCGATCATT	
tet1	F: GCAACCACTCCCATCCTTCGAC	KC689999
	R: TGTTAGCGACTGACGGGTAGGGAC	
tet2	F: CCAACAGTGACAGGACGAGG	KC690000
	R: TTGAATAGGGTGAGGGAGCAT	
tet3	F: CTGTCGGTTGGGATTGAAGGAAGG	KC690001
	R: TGTGGAGGTTATGCTGGTCTTTGTG	
β-actin	F: AGGTCATCACCATTGGCAAT	NM_131031
	R: GATGTCGACGTCACACTTCAT	

Table S2 Enriched biological processes related to reproduction and gametogenesis in the 50 μg/L group vs the control by GO analysis.

ID (BP)	**Term**	***p*-value**	**p_{adj}**	**Genes name**
GO:2000241	regulation of reproductive process	7.08E-15	8.57E-12	*zgc:173556*, *zp3a.2*, *zp3a.1*, *zp3c*, *zp2.1*, *zp3d.2*, *ENSDARG00000089966*, *zp2.3*, *zp2.6*
GO:0007339	binding of sperm to zona pellucida	8.42E-14	5.10E-11	*zgc:173556*, *zp3a.2*, *zp3a.1*, *zp3c*, *zp2.1*, *zp3d.2*, *ENSDARG00000089966*, *zp2.3*, *zp2.6*
GO:0035036	sperm-egg recognition	4.52E-13	1.37E-10	*zgc:173556*, *zp3a.2*, *zp3a.1*, *zp3c*, *zp2.1*, *zp3d.2*, *ENSDARG00000089966*, *zp2.3*, *zp2.6*
GO:0080154	regulation of fertilization	4.52E-13	1.37E-10	*zgc:173556*, *zp3a.2*, *zp3a.1*, *zp3c*, *zp2.1*, *zp3d.2*, *ENSDARG00000089966*, *zp2.3*, *zp2.6*
GO:0000003	reproduction	1.52E-11	2.62E-09	*zgc:173556*, *aspm, zp3a.2*, *slbp2*, *zp3a.1*, *zp3c*, *zp2.1*, *zp3d*.2, *ENSDARG00000089966*, *aurka*, *ENSDARG00000099801*, *cyp17a1*, *tdrd5*, *zp2.3*, *zp2.6*, *e2f5*, *fbxo5*, *dazl*, *fbxo43*, *cdc25b*

GO:0022414	reproductive process	1.52E-11	2.62E-09	*zgc:173556*, *aspm*, *zp3a.2*, *slbp2*, *zp3a.1*, *zp3c*, *zp2.1*, *zp3d.2*, *ENSDARG00000089966*, *aurka*, *ENSDARG00000099801*, *cyp17a1*, *tdrd5*, *zp2.3*, *zp2.6*, *e2f5*, *fbxo5*, *dazl*, *fbxo43*, *cdc25b*
GO:0009566	fertilization	1.72E-10	2.32E-08	*zgc:173556*, *zp3a.2*, *zp3a.1*, *zp3c*, *zp2.1*, *zp3d.2*, *ENSDARG00000089966*, *zp2.3*, *zp2.6*
GO:0019953	sexual reproduction	4.63E-09	4.66E-07	*zgc:173556*, *zp3a.2*, *slbp2*, *zp3a.1*, *zp3c*, *zp2.1*, *zp3d.2*, *ENSDARG00000089966*, *ENSDARG00000099801*, *tdrd5*, *zp2.3*, *zp2.6*, *e2f5*, *dazl*
GO:0044703	multi-organism reproductive process	4.63E-09	4.66E-07	*zgc:173556*, *zp3a.2*, *slbp2*, *zp3a.1*, *zp3c*, *zp2.1*, *zp3d.2*, *ENSDARG00000089966*, *ENSDARG00000099801*, *tdrd5*, *zp2.3*, *zp2.6*, *e2f5*, *dazl*

Note: false discovery rate (FDR) corrected *p*-value (p_{adj}) < 0.05 was considered significant.

Table S3 Enriched pathways related to reproduction and gametogenesis in the 50 μg/L group vs the control by KEGG analysis.

ID	**Description**	***p*-value**	p_{adj}	**Genes name**
dre00100	Steroid biosynthesis	1.25E-06	1.60E-04	*cyp24a1*, *sc5d*, *soat2*, *cyp2r1*, *msmo1*, *ebp*, *sqlea*
dre04914	Progesterone-mediated oocyte maturation	3.95E-04	1.68E-02	*cpeb1b*, *ccnb2*, *ccnb1*, *plk1*, *aurka*, *ccna1*, *ccna2*, *mos*, *mad2l1*, *cdc25b*, *ccnb3*
dre04114	Oocyte meiosis	2.76E-03	8.84E-02	*cpeb1b*, *ccnb2*, *ccnb1*, *plk1*, *aurka*, *mos*, *fbxo5*, *mad2l1*, *cdc20*, *fbxo43*
dre00140	Steroid hormone biosynthesis	1.53E-02	1.40E-01	*ugt1a2*, *cyp17a1*, *ugt5a2*, *cyp3c3*, *cyp11a1.1*

Note: false discovery rate (FDR) corrected *p*-value (*padj*) < 0.05 was considered significant.