REPRODUCTIVE AND HORMONE HOMEOSTASIS EFFECTS IN YUCHENG MEN EXPOSED TO PCBS/PCDFS

Hsu PC[1], Guo YL[2], Lambert GH[3]
[1]Department of Safety, Health and Environmental Engineering, National Kaohsiung First University of Science and Technology, Kaohsiung, Taiwan; [2]Department of Environmental and Occupational Medicine, National Taiwan University, Taipei, Taiwan; [3]Center for Child and Reproductive Environmental Health, Department of Pediatrics, Environmental and Occupational Health Service Institute, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, NJ, USA

Abstract
This follow-up study investigated reproductive effects of young boys and adults from a major polychlorinated biphenyls (PCBs) and their pyrolytic product, mainly polychlorinated dibenzofurans (PCDFs) exposure episode, which occurred in Taiwan between 1978 and 1979, referred to as "Yucheng" exposure. In 1995, 60 Yucheng and 61 control boys participated in physical examination, and serum hormones were measured by radioimmunoassay (RIA). The serum estradiol (E_2) levels were significant higher in Yucheng boys at the age of puberty. There was a decrease of serum testosterone (TT) levels and increase of serum follicle-stimulating hormone (FSH) levels in Yucheng boys at the age of puberty as compared with controls. In 1998, we found that sperm motility, morphology, asthenospermia rate, curvilinear velocity, average path velocity and straight-line velocity for motile sperm, and sperm capability of binding and penetration were significantly affected in prenatal exposed young boys. From 1999-2002, we found that PCBs/PCDFs postnatally exposed adults revealed higher percentage of abnormal morphology and prevalence of oligospermia than controls. The ability of sperm to penetrate hamster oocytes was significantly reduced in exposed men. How PCBs/PCDFs reprogram the germ line and to promote a transgenerational disease for environmental and occupational health warrants further investigation.

Introduction
Polychlorinated biphenyls (PCBs) and dibenzofurans (PCDFs) are persistent environmental pollutants that induce a broad spectrum of toxic effects in mammalian species.[1] Over 2000 Taiwanese people in 1978-79 ingested rice oil accidentally contaminated with PCBs and PCDFs.[2] They developed chloracne, hyperpigmentation, peripheral neuropathy, and other signs and symptoms. Children of Yucheng mothers transplacentally exposed to the toxicants had intrauterine growth retardation, dysmorphic and hyperpigmented skin and nails, and reduced neurocognitive development.[3] Animal study suggested that prenatal exposure to endocrine disruptors might produce adverse effects on male pubertal development via the changes of sex hormone levels.[4] However, epidemiological information concerning the effects of PCBs/PCDFs prenatal or postnatal exposure on reproductive functions and hormone homeostasis is still limited.

Materials and Methods
Yucheng boys: Yucheng children were born between 1979 and 1985, to women who had exposed to high doses of PCBs/PCDFs through consumption of contaminated rice bran oil in 1978-1979. In 1995, 60 Yucheng and 61 control boys participated for hormone levels analysis, including serum testosterone (TT), estradiol (E_2), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL). Yucheng and control boys were further divided into two subgroups according to before (age < 13 yrs) and at the age of puberty (age $\geq$ 13 yrs). To determine whether exposure to PCBs/PCDFs in utero alter reproductive functions in the human male, a total of 12 young men prenatally exposed to PCBs/PCDFs, 9 unexposed matched controls, and 14 volunteers from local school participated in the examination of semen analysis in 1998.
Yucheng Adults: In 1999-2002, postnatally exposed men and their controls aged 37-50 years were recruited for physical examination followed by semen analysis. A total of 40 Yucheng adults and 28 controls participated in the study. All participants were instructed to abstain from ejaculation for at least 4 days prior to providing semen specimens. Evaluations of sperm counts, morphology, motility, velocity were measured according to World Health Organization (WHO) guidelines (1992).[5] Sperm - hamster oocyte penetration was assessed. All interview, examination, and laboratory tests were done in a blinded fashion.

Results and Discussion

Yucheng boys: There was a decrease of serum TT levels and increase of serum FSH levels in Yucheng boys at the age of puberty as compared with controls (Table 1). There was a significant decrease of the square root of TT/E_2 and TT/FSH, however, the square root of E_2/FSH was increased in Yucheng boys at the age of puberty as compared with controls (Table 1). Data indicated that prenatal exposure to PCBs/PCDFs might have implications for boys' sex hormone homeostasis at puberty. Percentage of normal morphology was reduced in sperm of Yucheng boys (Table 2). Percentage of motile sperm and rapidly motile sperm were reduced. Among motile sperm, exposed boys had more sluggish velocity and beat cross frequency but similar straightness of movements as measured by computer-assisted sperm analysis (Table 2).

Yucheng Adults: Exposed men were found to have higher sperm morphological abnormality and oligospermia rate than controls (Table 2). Semen volume, sperm count, motility, velocity, amplitude of lateral displacement, and beat cross frequency were similar in sperm of exposed and control men. The ability of sperm to penetrate hamster oocytes and number of sperm bound to hamster oocyte, however, were significantly reduced in exposed men.

Discussion: These findings are compatible with animal studies in which prenatal as well as postnatal exposure to dioxin-lik or non-dioxin like PCBs reduced sperm function.[6-9] Recent epidemiological studies have suggested that low-level exposure to PCBs, such as may be experienced by the general population, have been associated with effects on sperm motility, sperm concentration, and total sperm count.[10-11] In this study, sperm findings in postnatal exposure study were similar to the second generation young men prenatally exposed to PCBs/PCDFs in reduced normal morphology and reduced capability of oocyte binding and penetration. The prenatally exposed young men had additional problems of reduced motility in sperm. In addition, the offspring gender ratio (male/female) was reduced in Yucheng men exposed to PCBs/PCDFs before age 20 years.[12] In conclusion, we have investigated that prenatal or postnatal exposure to PCBs can alter hormone homeostasis and/or reproductive function in sexually mature men and offspring in puberty. How PCBs reprogram the germ line and to promote a transgenerational disease for environmental and occupational health warrants further investigation.

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Table 1. Levels of sex hormones between prenatal PCB/PCDF-exposed (Yucheng) boys and unexposed controls in the subgroups of all age, before and at the age of puberty.

Sex Hormones	Control	Yucheng
Testosterone (TT, ng/mL)		
All age	2.2 ± 2.4 (n=47)	1.8 ± 2.1 (n=49)
Before puberty [a] (age < 13 yrs)	1.0 ± 1.5 (n=30)	1.3 ± 1.6 (n=34)
Puberty [b] (age ≥ 13 yrs)	4.2 ± 2.2 (n=17)	3.0 ± 2.4 (n=15)
Estradiol (E_2, pg/mL)		
All age	14.9 ± 11.1 (n=45)	23.7 ± 35.7 (n=49)
Before puberty [a] (age < 13 yrs)	11.0 ± 7.6 (n=28)	12.6 ± 14.6 (n=34)
Puberty [b] (age ≥ 13 yrs)	21.3 ± 13.2 (n=17)	48.6 ± 53.9 (n=15)*
$\sqrt{(TT/E_2)}$		
All age	0.4 ± 0.2 (n=45)	0.3 ± 0.2 (n=49)
Before puberty [a] (age < 13 yrs)	0.3 ± 0.2 (n=28)	0.3 ± 0.2 (n=34)
Puberty [b] (age ≥ 13 yrs)	0.5 ± 0.2 (n=17)	0.3 ± 0.2 (n=15)*
Follicle-stimulating hormone (FSH, mIU/mL)		
All age	3.4 ± 1.6 (n=45)	3.6 ± 1.8 (n=49)
Before puberty [a] (age < 13 yrs)	3.3 ± 2.0 (n=28)	3.3 ± 1.5 (n=34)
Puberty [b] (age ≥ 13 yrs)	3.4 ± 0.8 (n=17)	4.6 ± 2.2 (n=15) [#]
$\sqrt{(TT/FSH)}$		
All age	0.5 ± 0.4 (n=45)	0.6 ± 0.4 (n=49)
Before puberty [a] (age < 13 yrs)	0.5 ± 0.4 (n=28)	0.8 ± 0.5 (n=34)
Puberty [b] (age ≥ 13 yrs)	1.1 ± 0.3 (n=17)	0.8 ± 0.5 (n=15) [#]
$\sqrt{(E_2/FSH)}$		
All age	2.2 ± 1.1 (n=45)	2.3 ± 1.2 (n=49)
Before puberty [a] (age < 13 yrs)	2.0 ± 1.2 (n=28)	2.0 ± 0.9 (n=34)
Puberty [b] (age ≥ 13 yrs)	2.4 ± 0.8 (n=17)	3.1 ± 1.4 (n=15) [#]
Luteinizing hormone (LH, mIU/mL)		
All age	1.8 ± 1.2 (n=45)	1.9 ± 1.2 (n=49)
Before puberty [a] (age < 13 yrs)	1.5 ± 1.2 (n=28)	1.5 ± 1.0 (n=34)
Puberty [b] (age ≥ 13 yrs)	2.4 ± 1.0 (n=17)	2.6 ± 1.5 (n=15)
Prolactin (PRL, ng/mL)		
All age	0.3 ± 0.3 (n=45)	0.4 ± 0.3 (n=49)
Before puberty [a] (age < 13 yrs)	0.4 ± 0.3 (n=28)	0.4 ± 0.4 (n=34)
Puberty [b] (age ≥ 13 yrs)	0.3 ± 0.1 (n=17)	0.4 ± 0.3 (n=15)

[a] Before the age of puberty: Their mothers were pregnant after 1981 with duration of exposure to PCBs/PCDFs large than 2 years. [b] At the age of puberty: Their mothers were pregnant before 1981 with duration of exposure to PCBs/PCDFs less than 2 years. [#]: $0.05 < P < 0.1$; *: $P < 0.05$; Limit of detection: TT: 0.04 ng/mL; E_2: 8 pg/mL; FSH: 0.06 mIU/mL; LH: 0.15 mIU/mL; PRL: 0.1 ng/mL

Table 2. Comparison of age, physical examination, history relevant to reproductive system, and semen parameters between men prenatally and postnatally exposed to PCBs/PCDFs and unexposed controls.

Category	Prenatal exposure (Yucheng boys)		Postnatal exposure (Yucheng adults)	
	Ratio (Exposed / Control)	*P* values	Ratio (Exposed / Control)	*P* values
Age (yrs)	0.98	n.s.	0.94	n.s.
Body weight (kg)	0.96	n.s.	1.0	n.s.
Body height (cm)	0.98	n.s.	1.0	n.s.
Cigarettes smoking rate (%)	1.15	n.s.	0.95	n.s.
Frequency of erection (per day)	0.68	n.s.	1.10	n.s.
Conventional analyses of semen quality				
Semen volume (ml)	0.79	n.s.	0.86	n.s.
Sperm count (10^6/ml)	0.97	n.s.	0.96	n.s.
Motility (%)	0.61	0.006	0.97	n.s.
Abnormal morphology (%)	1.45	<0.0001	1.18	0.041
Oligospermia rate [a] (%)	1.0	n.s.	6.25	0.039
Asthenospermia rate [b] (%)	8.62	0.011	1.17	n.s.
Computer-assisted sperm analysis (CASA)				
VCL (μm/s)	0.76	0.007	1.12	n.s.
VAP (μm/s)	0.77	0.010	1.14	n.s.
VSL (μm/s)	0.77	0.018	1.16	n.s.
ALH (μm)	0.88	n.s.	1.24	n.s.
BCF (Hz)	0.81	n.s.	0.97	n.s.
Hamster oocyte penetration				
Percent of oocytes penetrated after 2 hr *in vitro* insemination	0.90	0.017	0.50	0.0004
Number of human sperm bound/hamster oocyte	0.81	0.010	0.59	0.0001

n.s.: not significant; [a]:sperm count < $20x10^6$ cells/ml; [b]: sperm motility < 50%; VSL: straight-line velocity; VCL: curvilinear velocity; ALH: amplitude of lateral displacement; BCF: beat frequency.