



Polychlorinated biphenyls and dibenzofurans increased abnormal sperm morphology without alterations in aneuploidy: The Yucheng study



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HIGHLIGHTS

- The study cohort has been exposed to high doses of dioxin-like chemicals.
- Abnormal morphology was found in sperm among Yucheng men.
- Sperm chromosome aneuploidy was not elevated among Yucheng men.
- Disproportional reduction of normal Y sperm was not observed in Yucheng men.

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ABSTRACT

In 1979, more than 2000 persons ingested rice oil contaminated with polychlorinated biphenyls and polychlorinated dibenzofurans; this event was called the “Yucheng accident.” An increased percentage of oligospermia, reduced ability of sperm to penetrate oocytes, and reduced percentage of male offspring were reported in Yucheng men. This study examined whether the sperm sex ratio and chromosome aneuploidy are responsible for our observed findings in Yucheng men. In 1999–2000, Yucheng men and their neighborhood referents aged 37–50 years were recruited for physical examination, followed by semen analysis. The semen samples were analyzed for chromosomal aneuploidy through fluorescent *in situ* hybridization according to an established procedure in our laboratory. A total of 50 Yucheng men and 34 neighborhood referents volunteered to participate in the study. Although abnormal morphology was mildly increased, no differences were observed in sperm percentages, with normal numbers of chromosomes X, Y, and 8 in the two groups. The percentage of sperm with aneuploidy of the sex chromosomes or chromosome 8 and of that with diploidy did not vary between both groups. The normal X/Y sperm ratio was not different between the groups. However, among Yucheng men, 8% had a normal X/Y sperm ratio of >1.4, and no neighborhood referent showed such an elevated X/Y ratio. Chromosomal aneuploidy was not elevated in Yucheng men. The mechanisms underlying the reduced sperm capability of oocyte penetration and changed offspring sex ratio in Yucheng men remain undetermined.

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1. Introduction

Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs), and other dioxin-like chemicals are ubiquitous food contaminants in developed countries around the world. Exposure to POPs has been

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linked to adverse effects on the male reproductive system (Toppari et al., 1996). A growing body of evidence has shown that paternal exposure to POPs has adverse effects on sperm parameters (Hauser et al., 2003; Hsu et al., 2003; Richthoff et al., 2003). Furthermore, studies have reported an increased proportion of girls born subsequent to paternal exposure to POPs (Mocarelli et al., 1996; 2000; del Rio Gomez et al., 2002).

During 1978–1979, more than 2000 Taiwanese persons ingested rice oil contaminated with PCBs and their pyrolytic products, mainly PCDFs. This event was referred to as the “Yucheng accident” (oil disease in Chinese) (Hsu et al., 1985). Studies have reported more chloracne, hyperkeratosis, abnormal nails, and skin allergies in this group than in the background exposure groups (Guo et al., 1997) and reviewed the health effects (Guo et al., 2003). The Yucheng population experienced an almost unparalleled exposure to PCBs and PCDFs. The exposure levels to these chemicals were similar to that in the Japanese Yusho population, which experienced a similar event in 1968, 11 years before the Yucheng event. Serum levels of dioxin-toxic equivalency (TEQ) in people who experienced the Yucheng accident (Guo et al., 1997) were higher than those in individuals of the Seveso population in Italy, who were exposed to tetrachlorodibenzodioxin (TCDD) (Eskenazi et al., 2004). Among men heavily exposed to PCBs and PCDFs, we observed an increased percentage of oligospermia, the reduced ability of sperm to penetrate oocytes (Hsu et al., 2003), and a reduced percentage of male offspring (del Rio Gomez et al., 2002).

The mechanism of such effects is unclear. Theoretically, the offspring sex ratio may be attributed to circumstances and events occurring before fertilization, including the sperm sex ratio. Regarding sperm functions, previous studies have indicated that sperm aneuploidy, particularly sex chromosome aneuploidy, is associated with severe damage to spermatogenesis (Johnson, 1998; Machev et al., 2005; Tempest and Martin., 2009). Based on this factor, this study examined whether the sperm sex ratio and sperm chromosome aneuploidy accounted for our observed findings in Yucheng men.

2. Materials and methods

2.1. Study population

This study was approved by the Institutional Review Board of the National Taiwan University Medical Center. We analyzed the Yucheng Registry (Hsu et al., 1985) and previously described neighborhood referents (Yu et al., 1997; Guo et al., 1999; Li et al., 2013). People who experienced the Yucheng accident were exposed to relatively high doses of PCBs and PCDFs. The average consumption was estimated to be 1 g (0.7–1.4) of PCBs and 3.8 mg (1.8–5.6) of PCDFs (Lan et al., 1981) among these people. The concentrations of PCBs and PCDFs TEQ in the blood of these people 16–17 years after the exposure were 133.4 ppt TEQ lipid and 315.1 ppt TEQ lipid, respectively (Lambert et al., 2006).

In 1999–2000, Yucheng men and their neighborhood referents aged 37–50 years were recruited for physical examination, followed by semen analysis. Complete medical history, occupational exposure, and reproductive history were obtained using a structured questionnaire. We excluded individuals with a history of reproductive disorders and those who had consulted medical doctors for reproductive problems.

2.2. Fluorescence *in situ* hybridization

Semen samples were analyzed for chromosomal aneuploidy through fluorescent *in situ* hybridization (FISH) with an established procedure in our laboratory (Kuo, 2002). The semen samples were

stored in liquid nitrogen after collection from the study participants and were transported to the Reproductive Toxicology Laboratory at National Cheng Kung University for the FISH study. Frozen semen specimens were thawed in a random order, mixed well, and 2 μ L was smeared onto premarked areas of clean glass microscope slides. After they had been dried at 60 °C for 2 h and fixed in a mixture of methanol and glacial acetic acid (3:1) overnight, the slides were pretreated with 0.2% Triton X-100 for 5 min at room temperature, followed by 10 mM DTT (Merck Co.) for 30 min at 47 °C and 4 mM lithium diiodosalicylate (LIS; Sigma Chemical) for 45 min at room temperature. These treatments decondensed the sperm head area by 1.5–1.8 times the original longitudinal, nuclear axis size to improve the efficiency of hybridization. The slides were subsequently air-dried and immediately subjected to the hybridization procedure. Direct-labeled fluorescence Vysis probes (Downers Grove, IL) CEP X (SpectrumAqua), CEP Y (SpectrumOrange), and CEP 8 (SpectrumGreen) were used for analysis of chromosomes X, Y, and 8 simultaneously in each sperm cell. The three centromere-specific probes recognized the α -satellite DNA of the tested chromosomes. One microliter of each probe was mixed with 56 μ L of hybridization buffer (Vysis) and 21 μ L of H₂O. Five microliters of the mixed solution was added onto the premarked area of each slide, and an 11 mm \times 11-mm coverslip was placed on the drop of the solution and sealed with rubber cement. The slides were transferred to the block of an automatic slide thermometer (HYBAID). DNA denaturation and hybridization were performed at 75 °C for 5 min and 37 °C for 3 h, respectively, in the same block. After hybridization, we discarded the coverslips and washed the slides in 50% formamide, 2 \times SSC, pH 7.0 (SSC = 0.15 M sodium chloride, 0.015 M sodium citrate) at 47 °C for 20 min, followed by 10-min wash in 2 \times SSC at 47 °C and in 2 \times SSC at room temperature. Once the slides were air-dried, 10 μ L of 125 ng/mL 4',6-diamidino-2-phenylindole (DAPI) in antifade mounting solution (Vysis) was added onto each slide and covered with a 22 \times 22-mm coverslip. The slides were stored at –20 °C in a light-free box until scoring.

2.3. Scoring for FISH

The slides were analyzed using an Olympus BX60 fluorescence microscope equipped with a WU filter for DAPI fluorescence and an MF filter for the simultaneous visualization of the SpectrumGreen, SpectrumOrange, and SpectrumAqua fluorescence. Moreover, 1000 decondensed and hybridized cells were scored for each specimen, and only cells that had a sperm shape, tail, or both were included in the scoring. Each cell was examined for the number of fluorescent spots, designating each of the specific chromosomes under study (X, aqua; Y, red; 8, green).

2.4. Statistical analyses

Statistical analysis was performed using JMP version 5.0 software. Data were summarized as mean \pm standard deviation for continuous variables and as total numbers and percentage for categorical variables. Differences in continuous and categorical variables were subsequently compared using t tests and chi-squared tests, respectively.

3. Results

A total of 50 men exposed to PCBs and PCDFs (Yucheng men) and 34 neighborhood referents volunteered to participate in the study. The age, body height and weight, and percentage of smokers were not different between both groups (Table 1). Moreover, the semen volume, sperm count, and percentage of motile sperm did not vary between Yucheng men and neighborhood referents

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