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Exposure to persistent organic pollutants and sperm sex chromosome ratio in men from the Faroe Islands

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ABSTRACT

People in the Arctic as well as fishermen on the polluted Swedish east coast are highly exposed to persistent organic pollutants (POPs). These compounds have been shown to affect the sperm Y:X chromosome ratio. In present study, the aim was to investigate whether polychlorinated biphenyl (PCB) congeners and 1,1,-dichloro-2,2,-bis(*p*-chlorophenyl)ethane (*p,p′*-DDE) influence sperm sex chromosome ratio in Faroese men, and whether these men differ regarding Y:X ratio compared to Greenland Inuit and Swedish fishermen.

The study population ($n = 449$) consisted of young men from the general population ($n = 276$) as well as proven fertile men ($n = 173$). The Y:X ratio was assessed by fluorescent in situ hybridization. Serum concentrations of POPs were measured using gas chromatography. Associations between POP concentrations and Y:X ratio were calculated using linear and non-linear regression models as well as trend analysis and pairwise comparison of exposure data categorized into quartiles.

The selected POPs were associated with Y:X ratio in fertile Faroese men, but not in the total population; *p,p′*-DDE (95% CI for $B = -0.005$ to -0.001, $p = 0.005$) and ΣPCB (95% CI for $B = -0.005$ to -0.001, $p = 0.012$). Since *p,p′*-DDE and ΣPCB correlated significantly ($r = 0.927$, $p < 0.001$), the results involving the exposure variables can be regarded as a single finding. The Y:X ratio for the total Faroese population was 0.500 ± 0.018, which was statistically significantly lower than in both Inuit and Swedish fishermen (0.512 for both).

In conclusion, Faroese men presented with lower Y:X ratio than Greenland Inuit and Swedish fishermen. Although no direct health effects are expected due to the lower Faroese Y:X ratio, it could be indicative of adverse effects on the reproductive system.

1. Introduction

Globally, the secondary sex ratio is 0.515 at birth, or 106 males being born for every 100 females (Pyeritz, 1998). Over the last five decades, several studies have shown that the birth sex ratio in many industrialized countries is decreasing. A Danish study showed a statistically significant decrease in the birth sex ratios for Sweden, Norway, Denmark and Finland from 1945 to 1998 (Moller, 1998). Another study found a statistically significant decrease in male/female birth ratio between 1950 and 1995 in the Netherlands, Canada and the United States (Davis et al., 1998), while a study from 2003 showed a male/female birth ratio decline in Europe and North America over the second half of the 20th century (Grech et al., 2003).

The underlying reason for the drop in the secondary sex ratio is unclear, but one theory states that environmental or occupational agents from anthropogenic sources affecting the male reproductive system in a negative manner could be part of the explanation (Toppari et al., 1996). An example of this is, for instance, the accident in Seveso, Italy in 1976, leading to the release of large amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and a subsequent decrease in the birth sex ratio in the offspring of men exposed prior to adolescence (Mocarelli et al., 1996, 2000). Also, following the Yucheng poisoning in Taiwan in 1979, exposure to polychlorinated biphenyls (PCBs) and dibenzofurans, impaired sperm

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quality and an increased proportion of girls being born was observed (Chen et al., 1985). Studies carried out in the Great Lakes area in the USA suggested that maternal exposure to PCB would lead to a decrease in the birth sex ratio (Weisskopf et al., 2003), while paternal exposure leads to increased birth sex ratio (Karmaus et al., 2002).

Theoretically, a skewed birth sex ratio could be due to a deviation in sex chromosome ratio in sperm cells. Previously, a positive association between serum concentrations of CB-153, the most abundant PCB congener, and 1,1,-dichloro-2,2,-bis(*p*-chlorophenyl)ethane (*p,p*′-DDE) and the proportion of Y-chromosome bearing sperm cells was found in a cohort of Swedish fishermen, whose exposure originated from consumption of fish from the polluted Baltic sea (Tiido et al., 2005). Also, a negative correlation between CB-153 and the Y-chromosome bearing fraction of sperm cells was found in a Polish cohort that served as a European background population with respect to pollutant exposure. However, no associations at all between certain persistent organic pollutants (POPs) and Y:X chromosome ratio were observed in Inuit men from Greenland, who are among the highest to be exposed in the world (Tiido et al., 2006). These contradictory results were explained as inter-country differences in total exposure profiles, but might to some degree be due to genetic or epigenetic differences as well.

p,p′-DDE is a metabolite of the pesticide *p,p*′-DDT while PCBs were produced for use in, among other things, carbon less paper, capacitors and transformers (Longnecker et al., 1997). Production of these chemicals has been banned in most countries. However, production of *p,p*′-DDT still takes place in certain areas, mainly in Africa, where it is used to fight malaria (Rogan and Chen, 2005). As these compounds are lipophilic and highly persistent to degradation, they easily bioaccumulate in adipose tissue and in the food chain (Jensen, 1987). Due to their semi-volatile nature they are able to migrate through cycles of evaporation and atmospheric cycling and thus be transported to locations great distances from where they were released (Ritter, 1995). Many of these compounds therefore cause contamination of Arctic food chains. Hence, populations like the ones in Greenland and the Faroe Islands whose diets traditionally consist of sea mammals, in which these chemicals bioaccumulate (Dam and Bloch, 2000; Fromberg et al., 1999; Weihe and Joensen, 2012) are heavily exposed (Grandjean et al., 2001; Jonsson et al., 2005; Petersen et al., 2007). Also, a diet heavy in fish from the contaminated Baltic sea has generated a population of POP exposed Swedish fishermen, in whom the exposure levels of CB-153 are comparable to that measured in Greenland Inuit (Jonsson et al., 2005).

Studies that measured serum POP exposure levels on the Faroe Islands have shown overall markedly higher concentrations than the average European population (Jonsson et al., 2005). For example, data on POPs exposure show that average serum concentrations of CB-153 on the Faroe Islands may be over ten-fold higher than in a general European population (Jonsson et al., 2005; Longnecker et al., 2003; Petersen and others, 2008) and the Faroese men are more highly exposed to *p,p*′-DDE (Petersen et al., 2007; Weihe and Joensen, 2012). In a previous study on Y:X chromosome ratio in sperm samples from Inuit on Greenland and Swedish fishermen, their Y:X ratios were both 0.512 (Tiido et al., 2006).

Based on this backdrop, we wished to investigate the impact of persistent organic pollutants on sperm sex chromosome ratio in Faroese men and secondly to compare the Faroese sperm sex chromosome ratio to the Swedish east coast fishermen and to the Inuit men.

2. Materials and methods

2.1. Subjects

Semen samples from Faroese men were obtained from three different sources: the UM, K1S and K5P cohorts. The UM cohort consists of young volunteers from the general population, born between 1981 and 1984. Of the 1023 men that were invited, 241 delivered a semen sample (participation rate 24%; see Supplemental material, Fig. S1) (Halling et al., 2013). The K1S cohort was generated from consecutive births at the three Faroese hospitals between 1986 and 1987 consisting of 510 boys and 512 girls. From this cohort 421 males were asked to participate in a semen study and the final sample population consisted of 240 of these men (participation rate 57%; see Supplemental material, Fig. S2) (Grandjean et al., 1992). The K5P cohort consists of men whose pregnant partner participated in a study focusing on fertility and environmental factors. Of the 376 males who were approached to participate in a semen study, 282 men consented, out of which 16 were excluded for various reasons, leaving a total of 266 proven fertile men in the study population (participation rate 71%; see Supplemental material, Fig. S3).

Blood and semen samples were collected at the same time for the UM and K5P cohorts, while semen samples from the K1S cohort were collected a year after the blood samples.

For this study, 212 semen samples from the UM cohort, 102 semen samples from the K1S cohort and 185 samples from the K5P cohort were available.

Of the total 499 semen samples available, 449 (90%) were included in the current study (181 from UM, 95 from K1S and 173 from K5P). Of the 50 remaining samples, 40 samples did not have enough cells for FISH and for 4 samples the FISH method failed. In addition, six samples were excluded from the analysis due to lack of exposure data.

In the comparison of all included and excluded samples, those men included were slightly older (mean = 28.7 years and 26.2 years, respectively; Table 1).

The men that were included also had higher sperm concentration (mean = 63.3 mill/mL; 21.7 mill/mL) than those who were excluded. There were no differences in POP serum concentrations between included and excluded men.

Regarding the subcohorts of the study group, the men in the UM cohort had significantly higher serum concentrations of *p,p*′-DDE than the men in the K1S cohort ($p < 0.001$). Also, the men in the K5P cohort, compared to the men in the K1S and the UM cohorts, were significantly older ($p < 0001$ for both) and had significantly higher serum concentrations of *p,p*′-DDE ($p < 0.001$ for both) and ΣPCB ($p < 0.001$ for both), respectively.

The project protocol was approved by the Faroese ethical review committee and the IRB at Harvard School of Public Health. Each subject provided written informed consent in accordance with the approved protocols.

For comparison, previously analyzed and reported FISH data of 161 Greenland Inuit and 155 Swedish fishermen (Tiido et al., 2006) were included in the current study to establish the possible differences in the Y:X chromosome ratio between the Faroese, Greenland Inuit and Swedish fishermen. These populations have been previously described (Tiido et al., 2006). In short, the Inuit were between 18.5 and 51.3 (mean 31) years old, proven fertile and had somewhat higher serum concentrations of *p,p*′-DDE than the Faroese cohort. The Swedish fishermen were between 23.8 and 67.5 (mean 47) years old and, in regard to exposure, had somewhat lower serum concentrations of *p,p*′-DDE than the Faroese. The birth sex ratios were similar in the Inuit, Swedish fishermen and the Faroese population: being 0.512, 0.515 and 0.517, respectively [CIA.gov].

2.2. Fluorescent in situ hybridization

Protein-nucleic acid (PNA) probes (DakoCytomation, Glostrup, Denmark) targeted against the centromere of the X chromosome (FITC-labeled) and the q-arm of the Y-chromosome (Rhodamine-labeled), respectively, were used for the FISH assay.

Semen samples were thawed at room temperature and 6 μL/sample was smeared onto positively-charged microscope slides (Superfrost Plus, Menzel Gläser, Braunschweig, Germany). The slides were left to air dry at room temperature for 24 h and subsequently nuclear DNA of