Environmental Research 138 (2015) 345–351

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

# Spermatogenic capacity in fertile men with elevated exposure to polychlorinated biphenyls

M.S. Petersen [a,*], J. Halling [a], P. Weihe [a], T.K. Jensen [b,d], P. Grandjean [b,c], F. Nielsen [b], N. Jørgensen [d]

[a] Department of Occupational Medicine and Public Health, The Faroese Hospital System, Tórshavn, Faroe Islands
[b] Department of Environmental Medicine, University of Southern Denmark, Odense, Denmark
[c] Department of Environmental Health, Harvard School of Public Health, Boston, MA 02215, USA
[d] Rigshospitalet, University Department of Growth and Reproduction, Copenhagen, Denmark

ARTICLE INFO

*Article history:*
Received 27 January 2015
Received in revised form
21 February 2015
Accepted 25 February 2015
Available online 10 March 2015

*Keywords:*
Semen quality
Reproductive hormones
Exposure
PCB
Fertile men
Faroe Islands

ABSTRACT

*Background:* Endocrine disrupting industrial chemicals, such as polychlorinated biphenyls (PCBs), are suspected to adversely affect male reproductive functions.
*Objectives:* The Faroe Islands community exhibits an unusually wide range of exposures to dietary contaminants, and in this setting we examined the possible association between PCB exposure and semen quality and reproductive hormones in fertile Faroese men.
*Methods:* Participants in this cross-sectional study include 266 proven fertile men residing in the Faroe Islands. PCB levels and hormone profiles were measured in serum samples taken at the clinical examination that included semen quality parameters.
*Results:* A significant positive association was seen between serum-PCB and the testosterone/estradiol ratio ($p=0.04$). In the unadjusted analyses, elevated PCB exposure was associated with increased serum concentrations of SHBG ($p=0.01$) and FSH ($p=0.05$). We found no association between the serum PCB concentration and the semen quality variables.
*Conclusion:* In this population of highly exposed fertile men, the current serum-PCB concentration was associated with higher androgen/estrogen ratio. Further studies are needed to establish the findings and further document PCB-associated hormonal effects, any time windows of increased susceptibility, and the role of PCB in sub-fecundity.



## 1. Introduction

Semen quality studies of fertile men have been undertaken in several parts of the world (Jorgensen et al., 2001; Swan et al., 2003; Iwamoto et al., 2013; Gao et al., 2008) showing regional differences. For example, a European study of 1082 partners of pregnant women reported the lowest semen quality in Danish men, followed by French and Scottish men, while Finnish men had the best (Jorgensen et al., 2001). Furthermore, studies of men from the general populations have shown that male sub-fecundity due to impaired semen quality is prevalent throughout Europe (Jorgensen et al., 2011, 2012; Paasch et al., 2008; Punab et al., 2002; Fernandez et al., 2012; Axelsson et al., 2011). A Faroese population-based study of 481 men showed low semen quality, around the same levels as Danish men (Halling et al., 2013). Although these descriptive studies have uncovered a prevalent sub-fecundity problem, the etiology is unclear and may include environmental chemicals. Endocrine disrupting industrial chemicals, such as polychlorinated biphenyls (PCBs), have been identified as a likely contributing factor (Meeker and Hauser, 2010; Sharpe, 2010; Vested et al., 2014). Recent experimental animal studies indicate that semen quality may be adversely affected by exposure during prenatal development as well as early postnatal life and even in adulthood (Sharpe, 2010; Vested et al., 2014; Dickerson et al., 2011). Epidemiological support is limited, as the most vulnerable time of exposure is unknown and may have occurred many years prior to the semen sampling (Skakkebaek, 2003). However, cross-sectional studies offer some support that increased body burdens of lipophilic pollutants, such as PCBs and the pesticide metabolite dichlorodiphenyldichloroethylene (*p,p′*-DDE), may be linked to abnormalities of human testicular function (Meeker and Hauser,

* Correspondence to: Department of Occupational Medicine and Public Health, The Faroese Hospital System, Sigmundargøta 5, Postbox 14, FO-110 Tórshavn, Faroe Islands.
*E-mail address:* maria@health.fo (M.S. Petersen).

http://dx.doi.org/10.1016/j.envres.2015.02.030
0013-9351/

2010; Vested et al., 2014; Cai et al., 2011).

Effects on semen quality may be mediated through interference with reproductive hormones. A subtle adverse effect on testicular function may be compensated by altered reproductive hormone stimulation of the testicles, and thus only reflected by altered levels of reproductive hormones necessary for spermatogenesis. Part of the reason for the apparent disagreement between studies may be that exposure parameters are measured at different time periods. Inclusion of subjects with high exposures and populations with wide exposure ranges is crucial to obtain sufficient statistical power to detect possible impact of pollutant exposures on semen quality. In this regard, the Faroe Islands is highly relevant, as an unusually wide range of pollutant exposures has been documented (Weihe et al., 1996). Increased exposures to PCBs and DDE in this fishing community are mainly due to the consumption of blubber from the pilot whale, which accumulates persistent lipophilic substances present in marine pollution (Bloch et al., 1990). Of note, the use of pesticides and related substances is very limited in the Faroes as there is no formal farming and these substances are only used in limited extend in private households. Our previous study of 438 PCB-exposed adolescents revealed inverse associations with serum concentrations of both luteinizing hormone (LH) and testosterone and a positive association with the sex hormone binding globulin (SHBG), while associations with other hormones, testicular size and Tanner stage were uncertain (Grandjean et al., 2012). Of note, no semen samples were collected in that study. We examined the possible association between pollutant exposure and reproductive function parameters, including semen parameters, in a population of fertile Faroese males.

## 2. Materials and methods

### 2.1. Study population

During the period January 2007 to September 2008, pregnant Faroese women participated in a project focusing on associations between fertility and environment. The consenting women were asked for permission to contact their male partners. If she agreed the partner received an invitation by mail. For men who consented, an appointment for examination was arranged. All the women delivered a live born child. Participation included delivery of one semen sample, venous blood sampling, a physical examination, and completion of a questionnaire. A total of 376 women who participated in the study agreed to provide name and address of their partners, who were then invited to the semen study, and 282 men volunteered. Within this group, five men were excluded because the pregnancy had been achieved by fertility treatment; eight were non-Faroese, and three men were not able to produce a semen sample. Thus, 266 fertile men were included (71% of all invited). The examination period for the men ranged from February 2009 to February 2010. Known diseases in reproductive organs or previous fertility treatments (not related to the current pregnancy) were recorded, but not considered exclusion criteria.

## 3. Physical examination

The men underwent a physical examination performed by one of two examiners. The examination included assessment of body weight and height, the Tanner stage of pubic hair, and any abnormalities of the penis, epididymis or testis including determination of the location of testis in scrotum. Testicular volumes were determined both by palpation using a Prader orchidometer (Lenz et al., 1993).

## 4. Questionnaire

A questionnaire was generated in Faroese based on that used in Denmark in a study of partners of pregnant women (Jorgensen et al., 2001; Jensen et al., 2001). The questionnaire included information on previous or current diseases, including any known history of fertility potential, time to pregnancy, some lifestyle factors like smoking and drinking habits. Questions on occupational exposure were not a part of the questionnaire, as occupational PCB exposure is not of concern in this population. The fathers filled in the questionnaire before the physical examination and responses were reviewed with the examining physician.

## 5. Semen samples

Semen samples were produced by masturbation, in most cases in a room next to the laboratory. However, five men produced the sample by masturbation at home and brought the samples to the laboratory within 30minutes. The examining physician asked the men about ejaculation abstinence period. The semen sample was analyzed according to the World Health Organization 1999 guidelines (World Health Organization W, 1999) modified according results from a study of inter-observer variation (Jorgensen et al., 1997). Semen volume was estimated by weighing the collection tube with the semen sample and subtracting the weight of the empty pre-weighed tube, while assuming that 1 mL semen equals 1 g. For sperm motility assessment, 10 μL of well-mixed semen was placed on a clean glass slide kept at 37 °C and covered with a $22 \times 22$ mm$^2$ coverslip. The preparation was placed on the heated stage of a microscope at 37 °C and immediately examined at 400 × magnification. The spermatozoa were classified as progressive motile (WHO class A+B), local motile (WHO class C) or immotile (WHO class D). Sperm concentration was determined using a Bürker-Türk haemocytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany). Total sperm count (semen volume × sperm concentration) was calculated. One technician performed all analyses. Morphology slides were made, Papanicolaou stained and finally assessed according to "strict criteria" (Menkveld et al., 1990) at the University Department of Growth and Reproduction (Department of GR) at the National Hospital (Rigshospitalet, RH) in Denmark by another technician. Technicians had no access to the identity or any other information about the study subjects.

## 6. Exposure assessment

On the day of the examination, a venous blood sample was drawn from each participant and centrifuged (3000*g*, 10 min). Serum was subsequently separated and kept frozen at–80 °C until it was analyzed. PCB concentrations in serum samples was determined at the University of Southern Denmark using solid-phase extraction, followed by dual column gas chromatographic analysis with micro-electron capture detection according to a method previously described (Petersen et al., 2006; Grandjean et al., 2012). The mono-*ortho* substituted congeners PCB 28, PCB 105, PCB 118 and PCB 156 and the di-*ortho* substituted PCB 52, PCB 101, PCB 153, PCB 138 and PCB 180, as well as other persistent compounds (*p,p′*-DDE, *o,p′*-DDT, HCH, β-HCH) were recorded.

Spiked quality control samples were included in each series of samples. The laboratory participates regularly and successfully in the German External Quality Assessment Program (G-EQUAS) for serum PCB analyses, coordinated by the University of Erlangen-Nüremberg, Germany. The results were adjusted for total serum lipid content and reported as μg per gram lipid. The total lipid