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Environmental contaminant levels and fecundability among non-smoking couples☆

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Abstract

Objective: To investigate the effects of low level maternal and paternal persistent contaminant exposures on fecundability among couples from the general population.
Methods: About 41 couples having their first pregnancy completed questionnaires and provided blood samples for analysis of metals, organochlorine pesticides, and polychorinated biphenyls. Associations of personal consumption and contaminant measures for mothers, fathers, and couples overall were analyzed through fecundability odds ratios (fOR, probability of pregnancy per month in more versus less exposed) in multivariable analyses.
Findings: Couples with higher reported caffeine consumption (couple consumption ≥111 drinks/month, fOR 0.25, 95% CI, 0.10, 0.63) and higher mercury concentrations in maternal blood (>1.2 μg/L or 0.24 ppm in hair, fOR 0.22, 95% CI, 0.07, 0.72) had lower fecundability, after adjustment for intercourse frequency.
Conclusion: Reduced fecundability at levels below the mercury reference dose warrants further research and prudent reduction in persistent toxic substances exposure among women and men of reproductive age.

Keywords: Fecundability; Environmental exposure; Mercury; Caffeine; Organochlorine insecticides; Polychlorinated biphenyls; Canada

1. Introduction

Widespread publicity on endocrine disruption by persistent bioaccumulative toxic substances has fuelled concerns about environmental contaminants as a potential cause of delayed fertility [1]. Human evidence on both infertility and delayed time to pregnancy was first associated with lifestyle exposures such as cigarette smoking [2] and caffeine consumption [3], though the latter has been questioned [4]. Occupational toxic exposures have included inorganic mercury (Hg) [5] pesticides [6] and lead (Pb), for both women [7] and men [8]. Reproductive risks from chemical contaminants present in the Great Lakes ecosystem have been postulated [9] but data indicating human reproductive health effects are limited. Higher infertility rates and lower fecundability in couples with relatively higher exposure to mixtures of polychlorinated biphenyls (PCB), organochlorine pesticides (OC) and metals (usually organic Hg) through sport fish consumption have been shown for men [10] and women [11,12].

Many studies use questionnaire-based estimates of exposures to link with fertility-related outcomes [13]. Except for blood lead and cotinine (for smoking), direct measurement of body bur-

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dens of persistent toxic substances has been limited. Jarell and colleagues [14] found persistent organochlorines in follicular fluid among in vitro fertilization (IVF) patients, with a positive association of hexachlorobenzene and cleavage rates. In further work, endosulfan levels were positively associated with irregular menstrual cycles in women [15]. Persistent organochlorines have been measured in seminal fluid [16].

We were unable to find research on couples not selected for particular occupational or environmental exposures, in which both parents' levels of persistent toxic substances were measured and linked to time to pregnancy. Our objective in the current research was to investigate effects of maternal and paternal measures of persistent toxic substances on time to pregnancy (TTP) among couples from a general population, taking into account other known factors affecting fecundability. A priori, we thought that metals and persistent organochlorine compound concentrations might be associated with reduced fecundability.

2. Methods

2.1. Setting and patients

We recruited couples through the Department of Obstetrics and Gynecology of St. Joseph's Hospital in Hamilton, a large urban hospital where approximately one-half of the city's births occur. Study nurses reviewed antenatal records submitted to the Labour and Delivery floor by the physician providing pre-natal care three months ahead of the expected date of confinement. They contacted the pregnant woman and ascertained whether the couple met the following inclusion criteria: woman up to 35 years old (to reduce variation in time to pregnancy due to age); woman's first third trimester pregnancy (to reduce variation in contaminant levels due to reductions via offspring and breastfeeding); and both partners non-smoking at the time of conception (to reduce the effect of cigarette smoking on time to pregnancy). Among couples recruited July 1996–December 1997, we preferentially asked roughly equal numbers of those conceiving in the first month and those taking longer than five months, and a few taking two to five months, to provide biological samples for laboratory analyses. Written informed consent was obtained from all mothers and fathers in accordance with hospital research ethics committee approval. Each member of each couple ($n = 41$) completed a questionnaire (most via self-report with some requiring study obstetric nurse assistance) and provided blood samples either prior to or at the time of delivery.

2.2. Questionnaire measures

The questionnaire included sections on: demographics (age, country of origin, language, etc.); work history (job type, etc.); environmental exposures (water source, sport fish consumption, etc.); personal consumption (food frequency, alcohol, caffeine etc.); relevant medical history (reproductive tract infections, trauma, disorders, etc.) and reproductive history (frequency of intercourse at time of conception, periods of abstention, pre-pregnancy height and weight, etc.) (Details available in [17]). To measure time to pregnancy (TTP), we used a simple question (adapted from [18–20]) asking the mother "Approximately how many months did it take you and your partner to become pregnant, starting from the menstrual period before you decided to become pregnant and/or started having unprotected intercourse? # of months". Such questions have been shown to have good reliability and validity for women up to a decade after conception [19].

2.3. Biological samples

Maternal and paternal blood samples were collected when the mother came in to give birth. For organochlorine pesticides (OC) and polychorinated biphenyls (PCBs) analysis, one blood sample was collected from the mother and father into a 10 ml EDTA-containing Vacutainer and gently everted several times for complete mixing. The sample was then transported to the laboratory and centrifuged to separate plasma from packed red blood cells. The plasma was transferred to Teflon-capped, glass vials (Supleco #2-3248) and stored at −20 °C until analysis. Lipid sample collection was similar except the plasma for lipid analysis was stored in Corning Cryovials at −70 °C until analysis. For metals analysis, blood was collected in an identical fashion but into a 7 ml Terumo Venoject II tube. For the metal samples there was no centrifugation and whole, well-mixed samples were transferred to polyethylene tubes and stored at −20 °C.

2.4. Laboratory analyses

Total lipids were extracted from freeze-dried plasma following a modification [21] of the Folch gravimetric technique [22]. A small aliquot of this lipid extract was saved for determination of lipid class composition by thin-layer chromatography coupled with flame ionization detection (TLC-FID) after the method of Parrish [23] on an Iatroscan MK-IV detector (Iatron Labs., Tokyo) equipped with SIII-chromarods (Ancal Inc). Lipids were also analyzed using chemical methods. Total and free cholesterol (TC and FC), triglycerides (TG) and phospholipids (PL) are individually measured using enzymatic methods on the Tecnicon automatic analyser (RA-500) with the following test-packs: Randox for TG and TC; BMC for FC and Wako for PL. Plasma total lipids were calculated from the chemical determination method using the summation method. The formula for summation was total lipids = 1.677 (TC-FC) + FC + TG + PL, as recommended by Patterson and colleagues [24].

Blood mercury levels were determined by cold vapor atomic absorption spectrometry using a Pharmacia instruments mercury monitor model 100 by the method of Ebbestadt and colleagues [25]. Prior to introduction into the AA spectrometer, 500 μL of blood was digested in a microwave with an equal volume of concentrated nitric acid. An aliquot of the digest was then introduced in the system's reaction chamber and the mercury vapor generated and detected. The precision of the instrument for inorganic Hg was 5% RSD at 9.43 μg /L and 4.4% RSD at 18.05 μg/L for organic mercury. The detection limit was 0.20 μg/L (1 nmol/L).

Blood lead levels were determined by graphic furnace atomic absorption spectrometry with Zeeman background correction (Perkin-Elmer model ZL 4100 [26]). Matrix matched calibration was performed using reference material from the CHUQ Inter-laboratory Comparison Program [27]. The detection limit was 1.0 μg/dL. Reproducibility, expressed as the coefficient of variation ($N = 10$, day-to-day) was 2.4, 2.3 and 5.0%, respectively, at level of 58, 25 y 6 μg/dL.

Blood plasmas were analysed for PCBs and other OCs based on those compounds likely to have adequate detection rates based on prior work in the Great Lakes basin [28,29] or for which there existed some concerns about reproductive effects. Organochlorine pesticides and their transformation products included were: aldrin, *cis*-chlordane, trans-chlordane, α-chlordane, γ-chlordane, pp′-DDE, pp′-DDT, α-endosulfan, β-endosulfan, hexachlorobenzene (HCB), ß-HCH hexachlorocyclohexane, mirex, cis-nonachlor, trans-nonachlor and oxychlordane. We measured PCB congeners 5 + 8,19, 28, 52, 66 + 95, 74 + 94, 99, 101, 105, 118, 138, 146 + 188, 153, 156 + 171, 170, 172, 177, 180, 183, 194, 196 + 203, 201, 202 + 157 and 206 (double numbers indicate co-elutions). Laboratory analyses were performed on an HP-5890 series II gas chromatograph with dual-capillary columns and dual Ni-63 electron-capture detectors in the laboratories of Jean-Philippe Weber of the Centre de Toxicologie (CHUQ), Institut National de Santé Publique du Québec, Québec City, Québec as per methods described earlier [29] and similar to those used by other teams [30,31]. Prior to chromatographic analysis, 2 ml of blood plasma were extracted with dichloromethane:methanol then cleaned-up on Florisil columns, and taken to a final volume of 100 mL. The sample was then injected into the chromatograph and peaks were identified by their relative retention times obtained on the two columns, using a computer program developed in-house at Centre de Toxicologie du Québec's. Quantitation was performed mainly on the Ultra-1 column. The detection limits of the method, based on 3 times the average standard deviation of noise are: 0.02 μg/L for chlorinated pesticides and PCB congeners (except for the really light such as 5 + 8 and 19 which had a limit of 0.04 μg/L); 0.04 μg/L for p, p′DDT and ß-HCH; 0.2 μg/L for total PCB as Aroclor 1260.