Analysis of PCB congeners (by GC-ECD) and dioxin-like toxic equivalence (by CALUX assay) in females with endometriosis and other fertility problems

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Introduction

Infertility affects 17% of the couples in Sweden, Britain and Denmark. In recent years, concern about possible female reproductive toxicity due to environmental contaminants, has been growing. Despite the extensive animal evidence of the adverse effects of persistent organic pollutants (POPs) on reproduction, there is paucity of information in humans. Recent studies in primates have demonstrated that chronic exposure to dioxin can induce endometriosis, consistent with the hypothesis that environmental exposures to POPs may play a role in the pathophysiology of this disease in humans [1].

In this study exposure assessment to POPs was performed by a combination of chemical and bioassay analysis in a selected female subpopulation. Major PCB congeners were quantified by chemical analysis using gas chromatography with electron-capture detection, whereas TEQ-values were determined through CALUX (chemical-activated luciferase gene expression) bio-assay. Furthermore, both exposure assessments were used to investigate the extent of POP contamination in biological tissues of medically diagnosed infertile females (e.g., endometriosis, idiopathic infertility), and whether a significant correlation exists between POP levels and the specific causes of infertility.

Materials and methods

Study design

The survey group included 101 women with medically-confirmed diagnosis of infertility. A couple is defined as *infertile* when conception has not been achieved after one year of unprotected sexual intercourse or the failure to deliver a live-born child [2]. All patients were currently undergoing treatment at one of the collaborating Centers for Reproductive Medicine spread over Belgium (University Hospital of Antwerp, Ghent, and Leuven), enrolled between 1995 and 1998. This study has been accepted by all Ethical Committees (Protocolnos. 96/44/107, 97/100, and ML 536, for UZA, UZG, and UZL respectively). All patients accepted their participation by signing an Informed Consent.

All women underwent laparoscopy (n=101) and in 42 cases endometriosis was diagnosed. The severity of the disease was classified according to the scalar scoring system of the American Fertility Society. For 20 women, no diagnosis was found after laparoscopy, and after reviewing their corresponding male medical records, eight of them were defined as idiopathic infertile. The control group comprised 25 women with mechanical infertility (tubal disease, tuboperitoneal factor, cervical factor, uterine factor). In these women no evidence of endometriosis was found at laparoscopy. All women with ovulatory dysfunctions (n=14) were excluded from further analysis. A questionnaire has been carried out, eliciting detailed personal data to ascertain risk factors for POP exposure and infertility.

Chemical and CALUX Analysis

Serum (n=101), adipose tissue (n=46) and follicular fluid (n=8) samples were taken for analysis. Major PCB congeners and chlorinated pesticides were quantified by chemical analysis using gas chromatography with electron-capture detection [3-5]. The TEQ-values were determined through CALUX bio-assay. Murk *et al.* [6] demonstrated that the CALUX (chemical-activated luciferase gene expression) assay is a rapid, sensitive assay for assessing the toxic potency of (mixtures of) Arylhydrocarbon Receptor (AhR)-active compounds in small aliquots of blood plasma. The CALUX responses for the extracts were converted into so-called CALUX TEQs (TCDD equivalents), using a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) standard curve.

Statistical Analysis

Statistical calculations to compare analytical results were performed by linear regression analysis, Pearson and Spearman rank correlation coefficients when appropriate. For infertility analysis, Odds Ratios (ORs) were calculated. A finding was considered statistically significant if its *p*-level was less than 0.05. All calculations described above were performed with Statistica (Version 5, 1997) procedures.

Results and Discussion

Comparison between CALUX assay and chemical analysis

Regression analysis reveals strong associations between serum and adipose tissue levels of PCB congeners from the same patient, both analysed by GC-ECD. The serum residues of CB-138, 153, 180 predict significantly the adipose tissue levels. Furthermore, PCB-correlations between levels in serum and follicular fluid from the same patient were strong, and statistically significant at $p < .05$.

In this study, exposure assessment was also performed using the CALUX-bioassay, which determines the total dioxin-like toxic equivalence of mixtures of POPs as present in body fluids, such as serum, and follicular fluid. Data of CALUX-TEQs were compared to chemically determined PCB congeners.
The results reveal that the CALUX TEQs in cleaned extracts correlated significantly with the sum of four major PCBs in serum (Table 1). These data indicate that the CALUX-bioassay may serve

as a simple, relative inexpensive pre-screening tool to be used for exposure assessment in epidemiological surveys.

Table 1: The sum of PCB congeners (ng/g lipid weight) and dioxin-like toxic equivalence (pg TEQ/g lipid weight)in Belgian female body fluids, and Spearman rank correlation coefficients between chemical and CALUX bioassay analysis in both of the biological matrices.

	sum PCBs[1,2] (GC-ECD)	CALUX-TEQs[2]	Correlation
Serum (n=101)	257.9 ± 105.0	53.1 ± 42.3	0.42 ($p < .001$)
Follicular Fluid (n=8)	192.5 ± 87.7	72.0 ± 39.8	0.79 ($p = .036$)

[1] sum PCBs = sum of four major congeners
[2] mean ± S.D.

Exposure assessment of POPs in female infertile patients

Preliminary results indicate that among the endometriosis patients 17% were found positive for dioxin-like compounds (cut-off: CALUX-TEQ > 100 pg TEQ/g serum lipid weight), compared to 4% in the controls. Consequently, endometriosis patients are 4 times more likely (OR 4.0, p= .182) to have high serum TEQ-levels compared to mechanical infertile women. Although the idiopathic infertile group was relatively small, 14% of them were positive for TEQ, hence they were 3.4 times more likely (OR 3.4, p= .432) to have high TEQ-levels compared to the control group. The statistical calculations using CB-138 serum levels, showed odds ratios in the same range for both of the case-control groups using 100 ng/g lipid weight as cut-off level (endometriosis OR 3.1, p= .136; idiopathic infertility OR 1.6, p= .568). Note that in these preliminary calculations, no adjustments were made for confounding factors as age, body mass index, smoking pattern, caffeine and alcohol consumption).

These statistical analyses indicate that both parameters (e.g., PCB congeners and CALUX-TEQs) can be used to evaluate exposure assessment in infertile females, though the CALUX results reveal more distinct associations with endometriosis.

Completion of the statistics is expected for June 1999. Therefore, the first complete scientific release of study findings will be during Dioxin'99. A complete copy of the study will be made available for Dioxin'99 attendees at the conference.

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