Human Reproduction, Vol.26, No.1 pp. 117–126, 2011
Advanced Access publication on November 24, 2010 doi:10.1093/humrep/deq312

human reproduction

ORIGINAL ARTICLE *Gynaecology*

Dioxins in ascites and serum of women with endometriosis: a pilot study

L.Y. Cai[1], S. Izumi[1,*], T. Suzuki[1], K. Goya[1], E. Nakamura[1], T. Sugiyama[1], and H. Kobayashi[2]

[1]Department of Obstetrics and Gynecology, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan
[2]Department of Clinical Pharmacology, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan

*Correspondence address. Tel: +81-463-93-1121; Fax: +81-463-91-4343; E-mail: s-izumi@is.icc.u-tokai.ac.jp

Submitted on December 16, 2009; resubmitted on October 7, 2010; accepted on October 13, 2010

BACKGROUND: Animal studies and laboratory experiments have demonstrated that exposure to dioxins may be involved in the pathophysiology of endometriosis. However, recent epidemiological investigations have shown conflicting results. Although peritoneal fluid is a specific microenvironment playing a pivotal role in the development of endometriosis, to our knowledge, there is no published study evaluating the concentrations of dioxins in serum and peritoneal fluid simultaneously. The present study explores the possible correlation between the local peritoneal fluid levels of dioxins and concurrent endometriosis.

METHODS: There were 17 infertile women enrolled in the present study. After the diagnostic laparoscopic examination, the women were divided into two groups: endometriosis ($n = 10$) and controls ($n = 7$). We measured 29 dioxins simultaneously in serum and peritoneal fluid samples: 7 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), and 12 polychlorinated biphenyls (dioxin-like PCBs). A dioxin toxic equivalency (TEQ) system was utilized to calculate the dioxin concentration in each sample.

RESULTS: Serum concentrations of itemized components of 29 dioxins were similar in the endometriosis patients compared with the controls. Higher concentrations of PCDFs and dioxin-like PCBs were observed in peritoneal fluid than in serum, whereas the reverse was shown for PCDDs. Statistical analysis showed that higher levels of dioxin TEQ (PCDDs and PCDFs) in peritoneal fluid were significantly associated with an increased risk of endometriosis (OR: 2.5; 95% CI: 1.17–5.34; $P = 0.035$).

CONCLUSIONS: This is the first report suggesting that higher concentrations of dioxins (PCDDs and PCDFs) in peritoneal fluid are linked to endometriosis. More detail and epidemiological research is warranted to further explore this link.

Key words: endometriosis / peritoneal fluid / PCDDs / PCDFs / dioxin-like PCBs

Introduction

Endometriosis is a common gynecological disorder characterized by the presence of endometrial tissue outside of the uterine cavity that causes pelvic pain and infertility with a 10% prevalence among fertile women (Strathy *et al*., 1982). The 'gold standard' for endometriosis diagnosis is direct visualization by laparoscopy or laparotomy. The exact prevalence of endometriosis in the general population is underestimated because most women are asymptomatic and therefore do not seek medical care (Balasch *et al*., 1996). Although the etiology and pathogenesis of this disease remain unknown, retrograde menstruation and implantation of endometrial cells into the peritoneum is the most widely accepted theory. Recently, a growing body of evidence has indicated that endometriosis is likely to be a multifactorial disease. A complex interaction between genetic factors, immunoinflammatory processes, cytokine activation and environmental factors is suggested to be involved in the disease etiology (Tariverdian *et al*., 2007; Rier, 2008).

In the past 15 years, attention has been drawn to investigate the association between the cause of endometriosis and environmental exposure to 29 dioxin and dioxin-like chemicals, namely: 7 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), 4 non-ortho and 8 mono-ortho coplanar polychlorinated biphenyls (dioxin-like PCBs) (Rier and Foster, 2002; Anger and Foster, 2008; Bruner-Tran *et al*., 2008). Rier *et al*. (1993) first reported that exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is associated with a dose-dependent increase in the incidence and severity of endometriosis in the rhesus monkey. Eight years later, they re-analyzed the blood samples from the first experiments and found the serum levels of dioxin-like PCBs were also increased in TCDD-treated animals with a high prevalence and severity of endometriosis, indicating that dioxin-like PCBs may play a possible role in the development of endometriosis (Rier *et al*., 2001). Further rodent animal studies demonstrated that treatment with dioxin or dioxin-like chemicals resulted in a dose-dependent enhancement in the size of surgically induced endometrial lesions

(Cummings et al., 1996; Johnson et al., 1997). Moreover, laboratory experiments have shown that dioxin exposure in explant cultures of human endometrial or stromal cells promotes CYP1A1 and CYP1B1 expression and inflammation-related chemokine secretion (Bofinger et al., 2001; Bruner-Tran et al., 2008; Yu et al., 2008). These findings support the evidence that dioxin and dioxin-like chemicals may contribute to the progression of endometriosis in rodents and non-human primates.

Dioxin and dioxin-like chemicals are mainly produced by industrial byproducts and garbage combustion processes. Owing to their lipophilic nature, these chemicals may be biomagnified in the food chain. A previous work has demonstrated that all 29 dioxin and dioxin-like chemicals show a high affinity for binding the aryl hydrocarbon receptor (AhR), also known as the dioxin receptor (Safe et al., 1998; Klinge et al., 1999; Ohtake et al., 2003; Igarashi et al., 2005). The ligated AhR rapidly forms a heterodimeric complex with the AhR nuclear translocator and moves to the nucleus, initiating a cascade of endocrine disrupting events which may promote endometriosis and other diseases (Safe et al., 1998; Klinge et al., 1999; Ohtake et al., 2003; Igarashi et al., 2005). For all 29 compounds with the ability to bind to AhR, the dioxin toxic equivalency (TEQ) for each compound was used to reflect their AhR-related biological potency based on an assess system using a toxic equivalency factor (TEF) relative to 2,3,7,8-TCDD (Van den Berg et al., 1998).

Specifically, the prevalence and the severity of endometriosis are increasing in developing countries because of increasing environmental pollution (Donnez et al., 2002; Arisawa et al., 2005). Animal studies and laboratory experiments have also demonstrated this link at the molecular level (Rier and Foster, 2002; Bruner-Tran et al., 2008). Nevertheless, the results of current epidemiological investigations of the association between dioxins and endometriosis remain controversial (Anger and Foster, 2008). To date, several case–control studies have suggested an association between endometriosis and exposure to dioxins and/or total PCBs (Mayani et al., 1997; Heilier et al., 2005; Louis et al., 2005; Porpora et al., 2006). In an Israeli study, 18% of the endometriosis patients were dioxin-positive when compared with 3% in the controls, indicating an association between TCDD and endometriosis (Mayani et al., 1997). Louis et al. (2005) observed a 3-fold increased risk of endometriosis for women in the third tertile of anti-estrogenic PCBs, suggesting that anti-estrogenic PCBs may be associated with the development of endometriosis (Louis et al., 2005). An Italian study found that concentrations of both dioxin-like and non-dioxin-like PCBs were significantly elevated in women with endometriosis (Porpora et al., 2006). A recent study in Belgium demonstrated that the serum TEQ levels of dioxin-like compounds were significantly increased in women with peritoneal endometriosis and those presenting with deep endometriotic nodules (Heilier et al., 2005), whereas one retrospective cohort study and two recent case–control studies have failed to find a significant association between dioxin exposure and endometriosis (Eskenazi et al., 2002; Tsukino et al., 2005; Niskar et al., 2009). Moreover, several reviews have pointed out a lack of consensus from studies of the link between endometriosis and dioxin exposure due to the different investigated areas, low sample sizes, methodological issues and different criteria for endometriosis diagnosis (Guo, 2004; Anger and Foster, 2008; Foster, 2008).

Peritoneal fluid is a specific microenvironment containing cellular populations and soluble factors, such as steroid hormones, cytokines, growth factors and angiogenic factors. Growing evidence suggests that peritoneal fluid may play a critical role in the implantation and progression of endometriosis (Koninckx et al., 1998). However, to our knowledge, all the human epidemiological studies to date have only focused on the serum concentrations or TEQ levels of dioxin and/or dioxin-like chemicals; there are no published studies investigating the peritoneal fluid concentrations of dioxins and their link with endometriosis. In order to explore the possible correlation between the local peritoneal fluid levels of dioxins and the presence of endometriosis, we investigated the TEQ level of 29 dioxin and dioxin-like chemicals both in serum and ascites samples from 17 infertile Japanese women either with or without endometriosis.

Materials and Methods

Patients and samples

Seventeen Japanese women undergoing diagnostic laparoscopy for infertility were enrolled in this case–control study from October 2004 to March 2007 in the Department of Obstetrics and Gynecology, Tokai University School of Medicine. Endometriosis was diagnosed by visualization of the peritoneal cavity, ovaries, outside of the fallopian tubes and uterus during laparoscopy according to the revised classification of the American Society of Reproductive Medicine (Revised American Society for Reproductive Medicine Classification of Endometriosis: 1996, 1997). Ten women were diagnosed as having endometriosis and seven without endometriosis composed the control group. The characteristics of the study population are listed in Table I. All the participants provided written informed consent to be included in this study before the laparoscopic examinations. The study protocols were approved by the Ethical Committee of Tokai University School of Medicine. Approximately 10–13 ml of serum and 10–52 ml of peritoneal fluid were collected from each participant in her follicular phase not during the menstrual period. The serum and the peritoneal fluid samples were stored at −80°C until the analyses.

Table I Characteristics of the study population.

	Endometriosis	Controls	*P*-value
n	10	7	
Age (years)[a]	33.5 ± 3.6	36.4 ± 5.9	0.22
BMI[a]	20.9 ± 2.3	22.8 ± 4.6	0.33
Current smoking (%)[b]	10%	14.3%	1.0
Children[b]			
0	9	7	1.0
⩾1	1	1	
Volume of serum (ml)[a]	10.8 ± 0.8	11.0 ± 1.1	0.67
Volume of ascites (ml)[c]	18.0 ± 10.3	21.8 ± 14.9	0.67

Data are presented as the mean ± SD.
[a]Student's *t*-test.
[b]χ^2-test.
[c]Mann–Whitney *U*-test.

Chemical analyses

In 2008, all serum and ascites fluid samples were analyzed using high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (GC/HRMS; an Agilent 6800 series GCs, Agilent Technologies, Inc., CA, USA; an Autospec Ultima NT, Micromass Ltd, Manchester, UK) for 29 dioxin and dioxin-like compounds, including 7 PCDDs (TCDD, 1,2,3,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, 1,2,3,6,7,8-hexaCDD, 1,2,3,7,8,9-hexaCDD, 1,2,3,4,6,7,8-heptaCDD and 1,2,3,4,6,7,8,9-octaCDD), 10 PCDFs [2,3,7,8-tetra-chlorodibenzofuran (CDF), 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,6,7,8-hexaCDF, 1,2,3,7,8,9-hexaCDF, 2,3,4,6,7,8-hexaCDF, 1,2,3,4,6,7,8-heptaCDF, 1,2,3,4,7,8,9-heptaCDF and 1,2,3,4,6,7,8,9-octaCDF], 4 non-ortho PCBs and 8 mono-ortho PCBs (International Union of Pure and Applied Chemistry Nos. 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189). The detailed analytical methods and quality-control procedures were referred to previously (Patterson *et al.*, 1987; Patterson *et al.*, 1990). For the lipophilic nature of dioxin and dioxin-like chemicals, the concentrations of these compounds were adjusted for the lipid content of the samples. The concentrations of total serum lipid were determined according to previous descriptions (Akutsu *et al.*, 2008). For peritoneal fluid samples, we first determined the mean content of lipid in peritoneal fluid from three individual samples using 10 g of the fluid. Then, 10 g of peritoneal fluid from each participant was drawn to determine the dioxin and dioxin-like compounds, and the total lipid in these peritoneal fluid samples was calculated based on the mean lipid content. The TEQ for each PCDD, PCDF and dioxin-like PCB in a serum sample and ascites fluid was calculated based on its serum and ascites fluid concentration multiplied by its respective TEF (Van den Berg *et al.*, 1998). Subsequently, a total TEQ for each serum and peritoneal fluid sample was calculated from these individual TEQs. A concentration below the limit of detection (LOD) was considered as undetermined and calculated as zero. LOD was defined as the value of three times the signal/noise ratio in our GC/HRMS system.

Dealing with non-detects in chemical analyses

In the present study, some participants showed values lower than the LOD of some dioxin compounds; in which cases, we assigned those values as zero. This could be underestimating the exact concentration of dioxin and dioxin-like compounds in our method. To properly deal with non-detects, there may not be a best method; some have assigned half of the LOD, whereas others have used the LOD/$\sqrt{2}$. In this context, we alternatively assigned half of the LOD as the value for samples below the LOD and compared those results with the system of using zero for values lower than the LOD. However, since we could not find any differences in the statistical results between the two systems and preferred to avoid overestimation of samples below the LOD, we decided to use zero as the value of samples below the LOD.

Statistical analyses

All data are shown as the mean $\pm$ SD. Statistical analyses were performed using Dr SPSS II for Windows (release 11.0.1J, SPSS Japan Inc., Tokyo). The χ^2-test, the Mann–Whitney *U*-test and Student's *t*-test were used for comparing the characteristics of endometriosis patients and controls as appropriate. Total levels of PCDDs, PCDFs and PCBs in serum and ascites were calculated for the sums of the serum and ascites molar concentrations of PCDDs, PCDFs and PCBs. The serum and ascites levels of PCDDs, PCDFs and PCBs were adjusted by the lipid content. Differences in LODs in the serum and ascites samples were compared using Student's *t*-test or the Mann–Whitney *U*-test as appropriate. A Pearson correlation or a Spearman rank correlation coefficient was used to measure co-linearity of LODs between serum and ascites samples. Unconditional logistic regression analysis was used to estimate the association between total TEQ of dioxin in ascites and endometriosis. A *P*-value of <0.05 was considered to indicate statistical significance. Receiver operating characteristic (ROC) curves were used to assess the power to detect a difference and to estimate the sample sizes to validate our final results ((Centor, 1991).

Results

A total of 17 patients including 10 endometriosis patients and 7 normal controls were enrolled in this study. Serum and peritoneal fluid were collected from all the participants to simultaneously determine the concentrations of 29 dioxins. Table I shows the characteristics of the 17 women. The endometriosis patients and the controls were well-matched regarding age, body mass index (BMI), parity and smoking habits; and the volumes of collected serum and peritoneal fluid were similar between the two groups.

Most of the 29 dioxins were detected in almost all of the serum samples (Fig. 1). The distribution patterns of PCDDs, PCDFs (Fig. 1A) and dioxin-like PCBs (Fig. 1B) compounds in serum were similar between the endometriosis patients and the controls. In the ascites samples, only five compounds of PCDDs and PCDFs (2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,6,7,8-hexaCDF, 1,2,3,4,6,7,8-heptaCDD and octaCDD) were detected (Fig. 2A), but all of the dioxin-like PCBs were observed with higher levels than those in serum (Figs 2B and 4). Table II shows the mean level of the total TEQ values of PCDDs, PCDFs and dioxin-like PCBs in both the serum and the ascites samples, and the summarized sample numbers over the LOD appear in brackets. The total TEQ values of PCDDs, PCDFs and dioxin-like PCBs are similar for the endometriosis patients and the controls, and there were no statistically significant differences. The total TEQ values of PCDDs were significantly decreased in ascites compared with those in serum (Fig. 3A). The reverse was observed for PCDFs, showing that there were higher total TEQ values of PCDFs in ascites (11.3 pg TEQ/g lipid) than those in serum (7.3 pg TEQ/g lipid), but there were no statistically significant differences (Fig. 3B). On the other hand, the dioxin-like PCBs were significantly increased in ascites (Fig. 4A–C).

We subsequently analyzed the association of the dioxin residues in serum with their concentrations in ascites. The TEQ of dioxin-like PCBs in serum samples showed a close correlation with that in ascites (Fig. 4D–F), but the TEQ of PCDDs and PCDFs in serum did not relate to that in the ascites samples (Fig. 3C and D). On the other hand, the TEQ of PCDDs showed a positive linear correlation with the TEQ of PCDFs in serum samples ($r = 0.92$, $P = 1.3 \times 10^{-7}$, Fig. 5A). The linear regression equation is: total TEQ of PCDDs $= 1.12 \times$ total TEQ of PCDFs $- 0.11$. Whereas, in the ascites samples, the TEQ of PCDDs showed a non-linear correlation with the TEQ of PCDFs (Fig. 5B). The distribution pattern of total TEQ of PCDDs in 17 samples was divided into two groups, a higher level group (>0.4 pg TEQ/pg lipid) and a lower level group (<0.4 pg TEQ/pg lipid), whereas the distribution pattern of total TEQ of PCDFs in 17 samples can be divided into detectable and non-detectable groups. Statistical analysis by the χ^2-test showed that the higher-level TEQ of PCDDs with detectable levels for TEQ of PCDFs in ascites was significantly linked with endometriosis (Fig. 5C;

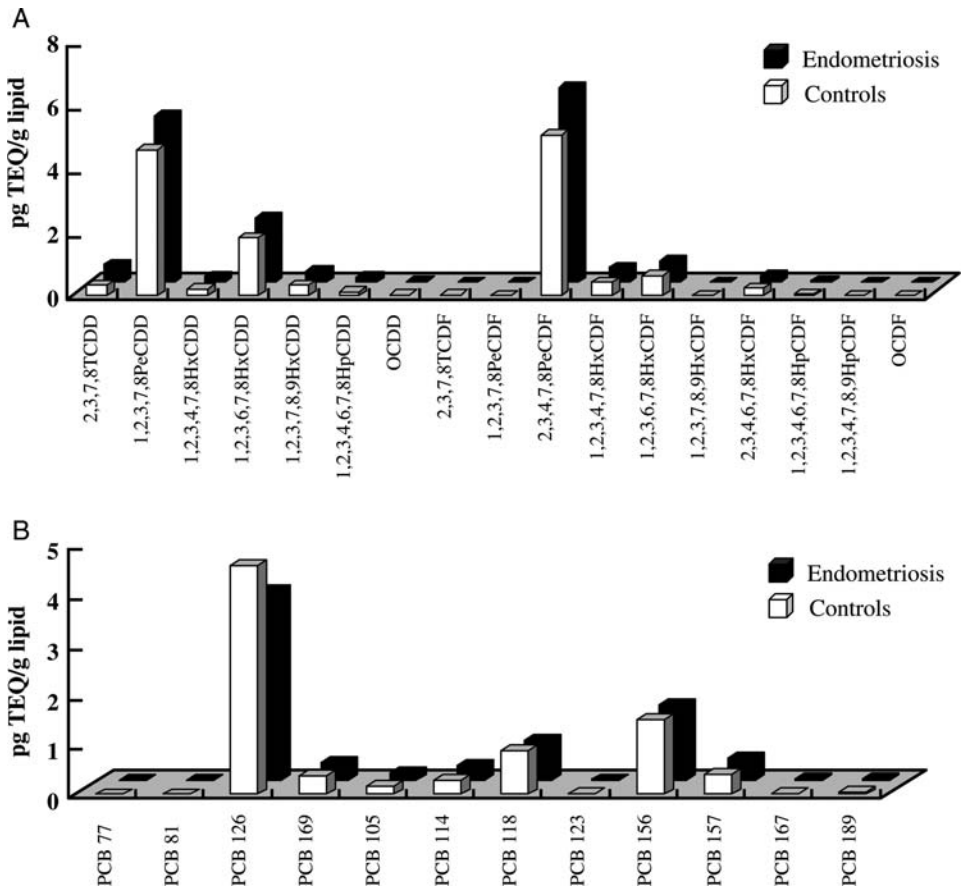

Figure 1 Distribution pattern of dioxins (**A**) and dioxin-like PCBs (**B**) in serum.

$P = 0.035$) where the odds ratio was 2.5 (endometriosis versus others; 95% confidence interval (CI): 1.17–5.34).

TCDD and other AhR agonists, including dioxin-like PCBs, have been shown to have anti-estrogenic or estrogenic activity, both through the mechanisms of crosstalk between AhR and estrogen receptor signaling pathways (Safe *et al.*, 1998; Ohtake *et al.*, 2003). Thus, the majority of the biologically adverse effects of dioxins and dioxin-like PCBs are through AhR-mediated mechanisms. Here, we made a comparison of the top three level PCBs (i.e. PCB 118, PCB 126 and PCB 156) according to their anti-estrogenic or estrogenic characteristics (Louis *et al.*, 2005) using the TEQ exposure metric (Van den Berg *et al.*, 1998). There was no significant difference between the endometriosis patients and the controls in the TEQ levels of ascites and serum PCBs based on their biological activity (Table III).

Discussion

It is still controversial whether dioxins are involved in the onset or development of endometriosis as endocrine disruptors (Rier and Foster, 2002; Anger and Foster, 2008; Bruner-Tran *et al.*, 2008). Animal models and laboratory studies have demonstrated that dioxin exposure may be involved in the pathophysiology of endometriosis, whereas epidemiological investigations remain inconsistent (Rier *et al.*, 1993; Cummings *et al.*, 1996; Johnson *et al.*, 1997; Guo, 2004; Foster, 2008). In the present study, we evaluated the concentrations of 29 dioxins in serum and peritoneal fluid simultaneously and explored the possible link between the ascites LODs and the concurrence of endometriosis. Our results show that in both serum and ascites samples the total TEQ LOD and dioxin-like PCBs were not significantly different between the endometriosis patients and the controls (Table II). The TEQ of PCDDs and dioxin-like PCBs in the serum samples showed a close correlation with that in ascites (Figs 3C and 4D–F); however, the TEQ of PCDFs in serum did not relate to that in the ascites samples (Fig. 3D). We also found that the TEQ of PCDDs showed a positive linear correlation with the TEQ of PCDFs in the serum samples (Fig. 5A), whereas the total TEQ of PCDDs and PCDFs in ascites showed distinct distribution patterns (Fig. 5B). There was a significantly increased number of patients with higher levels of both PCDDs and PCDFs in ascites in the endometriosis group compared with the control group [odds ratio (OR): 2.5 (1.17–5.34), $P = 0.035$, Fig. 5C].

PCDDs, PCDFs and dioxin-like PCBs are ubiquitous environmental pollutants that become concentrated in body fluids, such as plasma, breast milk and follicular fluid (Tsutsumi *et al.*, 1998; LaKind, 2007). To our knowledge, this study is the first to analyze concentrations of PCDDs, PCDFs and dioxin-like PCBs, a total of 29 chemicals, in both serum and ascites samples from endometriosis patients and

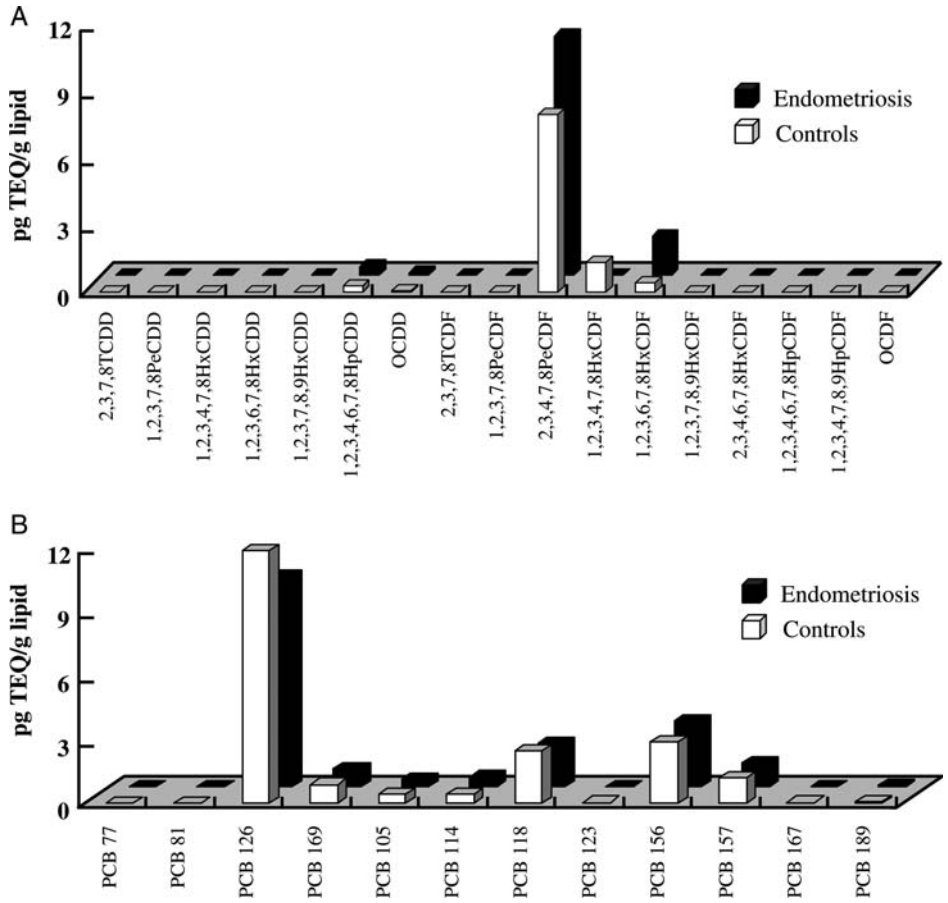

Figure 2 Distribution pattern of dioxins (**A**) and dioxin-like PCBs (**B**) in ascites.

Table II Lipid-adjusted levels of dioxin-like PCBs, PCDDs and PCDFs in serum and ascites.

	Serum levels			Ascites levels		
	EM (n = 10)	Control (n = 7)	P-value	EM (n = 10)	Control (n = 7)	P-value
PCDFs (10)	7.5 ± 2.6 (51/100)[a]	6.9 ± 2.3 (37/70)	0.61[b]	11.8 ± 10.4 (10/100)	10.6 ± 12.5 (6/70)	0.84[b]
PCDDs (7)	8.6 ± 3.2 (64/70)	7.4 ± 2.7 (34/49)	0.43[b]	0.5 ± 0.5 (15/70)	0.3 ± 0.4 (10/49)	0.89[c]
Total (PCDDs + PCDFs) (17)	16.1 ± 5.6 (115/170)	14.3 ± 3.1 (71/119)	0.51[b]	12.2 ± 10.5 (25/170)	10.8 ± 12.3 (16/119)	0.89[c]
Total non-ortho Co-PCBs (4)	4.1 ± 1.6 (31/40)	4.7 ± 3.2 (23/28)	0.81[c]	11.3 ± 3.9 (27/40)	12.1 ± 7.5 (20/28)	0.81[c]
Total mono-ortho Co-PCBs (8)	3.2 ± 0.6 (80/80)	3.1 ± 1.4 (56/56)	0.74[b]	7.4 ± 2.9 (80/80)	7.4 ± 2.5 (56/56)	0.86[b]
Total Co-PCBs (12)	7.2 ± 1.8 (111/120)	7.5 ± 3.9 (79/84)	0.54[c]	18.7 ± 5.3 (107/120)	19.3 ± 9.7 (76/84)	0.74[c]

Data are presented as the mean ± SD (pg TEQ/g lipid).
[a]Sample numbers over the LOD appear in brackets.
[b]Student's t-test.
[c]Mann–Whitney U-test.

controls. Because dioxins bioaccumulate over time, heavier women have higher body burdens because of the lipophilic nature of chemicals. Although apparently not statistically significant, the controls tended to be older (36.4 versus 33.5; controls versus patients, respectively) and have a higher mean BMI (22.8 versus 20.9; controls versus patients, respectively). This tendency might possibly mask the difference between the two groups. In this regard, we tried to find correlations between dioxins and BMI as well as age, but no correlations were found (data not shown). Congener profiles of PCDDs, PCDFs and dioxin-like PCBs in serum in the present study were

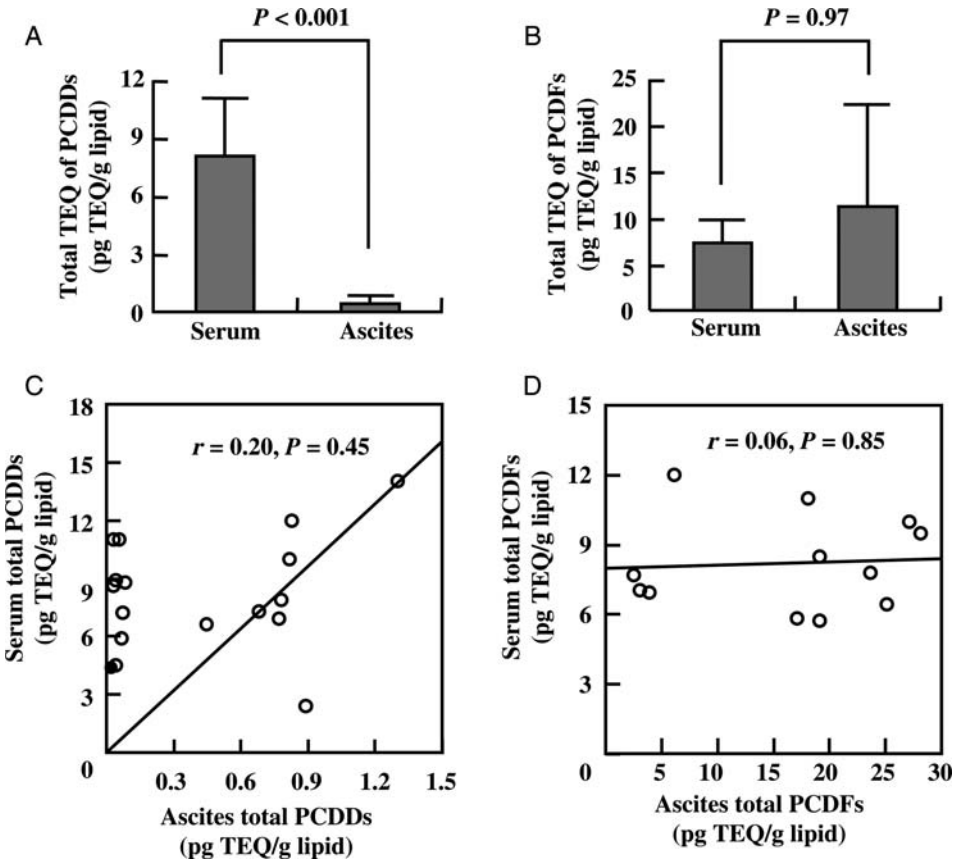

Figure 3 PCDDs and PCDFs in serum and ascites. When the total TEQ value was compared with serum and ascites samples, PCDDs in serum were higher than those in ascites (**A**) (0.4 pg TEQ/g lipid versus 8.0 pg TEQ/g lipid), whereas PCDFs showed the reverse tendency but without statistical significance (**B**). As for a linear correlation between the values of serum and ascites, no linearity was observed in either PCDDs (**C**) or PCDFs (**D**).

similar to those in a previous report (Fig. 1; Heilier *et al*., 2005). In our study, the estimated mean total TEQ serum value of PCDDs, PCDFs and dioxin-like PCBs was 23.3 pg TEQ/g lipid in the controls. This value is lower than those reported by two Belgian research groups in which the median values were 29.1 and 27 pg TEQ/g lipid (Pauwels *et al*., 2001; Heilier *et al*., 2005, respectively). Our results were slightly higher than those previously reported for other Japanese infertile women by Tsukino *et al*. (2005); they measured 25 PCDDs, PCDFs and PCBs, and the median total TEQ in the controls was 19.2 pg TEQ/g lipid, for which values lower than the LOD were assigned zero as was done in the present study. Another Japanese research group determined 29 dioxins and dioxin-like PCBs from randomly selected groups of 131 men and 122 women, and the median total TEQ serum values were 17 and 16 pg TEQ/g lipid, respectively (Arisawa *et al*., 2003), which were lower than those in the present study. These results could suggest that the dioxins and dioxin-like PCBs were concentrated to higher levels in infertile women and/or that the bioaccumulation of dioxins differ in the investigated areas.

In the present study, the estimated PCDDs level in ascites was significantly lower than in serum (Fig. 3A), and the explored levels did not show a significant association between ascites and serum (Fig. 3C). Although the total TEQ values of PCDFs in ascites were higher than in serum, but without statistical significance (Fig. 3B), the dioxin-like PCBs showed statistically significant higher levels in ascites than in serum with good correlation with each other (Fig. 4). Those results may indicate that the filtration pattern of PCDDs in ascites was different from that of PCDFs or dioxin-like PCBs (i.e. dilution in PCDDs and concentration in PCDFs or dioxin-like PCBs). More detailed research designed to disclose this mechanism is warranted.

Moreover, we found that simultaneous higher-levels of PCDDs and PCDFs in ascites were closely associated with endometriosis (Fig. 5C). The total TEQ value of PCDDs showed close association with the TEQ value of PCDFs in serum (Fig. 5A), but in ascites PCDDs and PCDFs showed distinct distribution patterns (Fig. 5B). Statistical analysis by the χ^2-test showed that the higher TEQ levels of PCDDs (higher than the mean concentration; 0.4 pg TEQ/g lipid) with detectable TEQ levels of PCDFs in ascites was a risk factor for the development of endometriosis [OR: 2.5 (1.17–5.34), $P = 0.035$). To our knowledge, the present study is the first to demonstrate that PCDDs and PCDFs in ascites are closely linked with endometriosis, especially when those levels were simultaneously high. More detailed and focused epidemiological research is warranted on this topic.

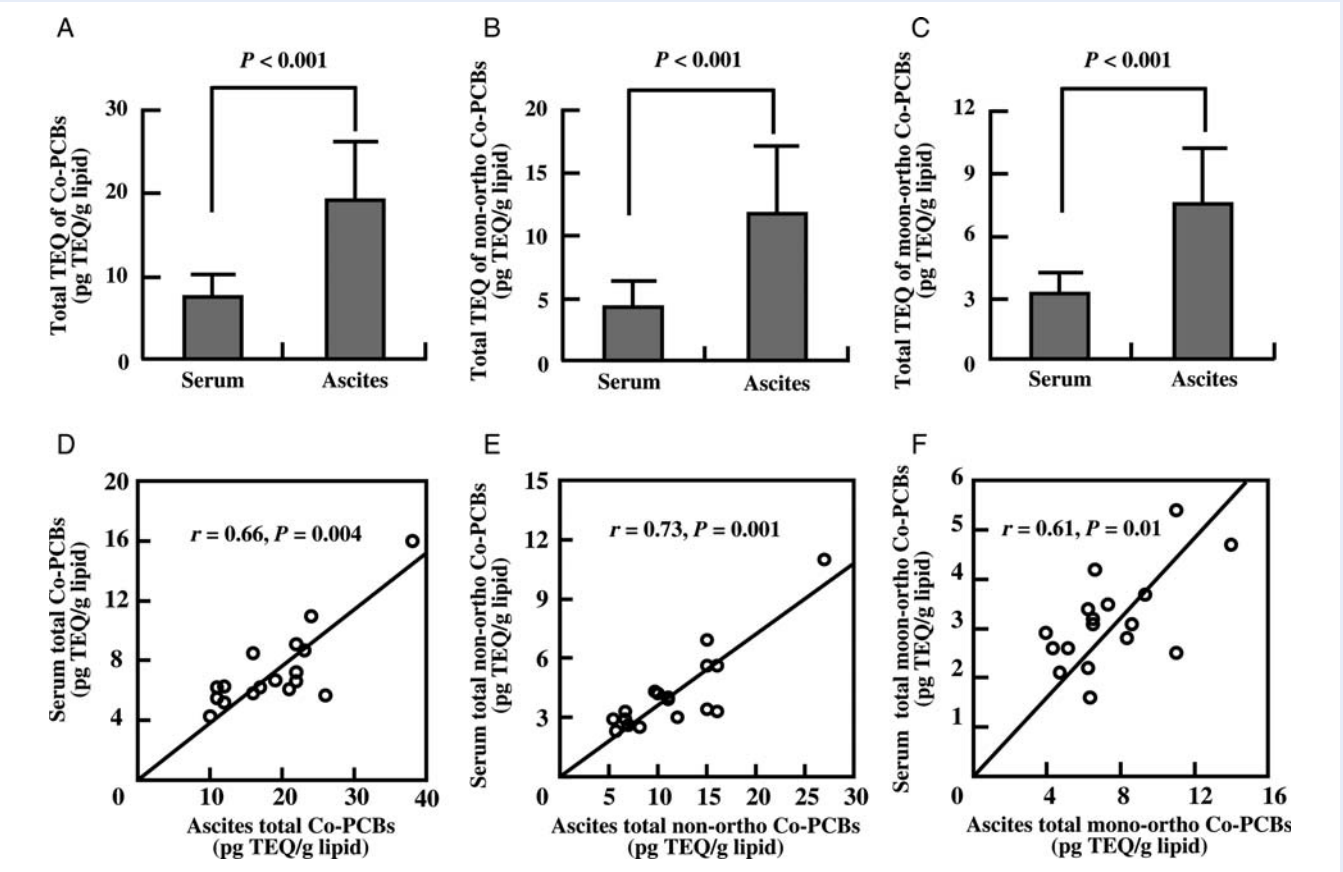

Figure 4 Dioxin-like PCBs in serum and ascites. When the total TEQ value of dioxin-like PCBs was compared with serum and ascites samples, the ascites level was significantly higher than the serum level (**A**), as it was in the non-ortho types (**B**) and the mono-ortho types (**C**). Regarding the linear correlation between the values of serum and ascites, linearity was observed (**D**) for total PCBs as well as for the two compartments: non-ortho types (**E**) and mono-ortho types (**F**).

The present study may be regarded as a pilot study considering the small sample size. A power analysis for these samples (10 and 7 samples from the positive endometriosis and negative control groups, respectively) using an ROC curve yielded 0.58 power (i.e. underpower) to detect a difference of 0.3 between the area under the ROC curve (AUC) for this analysis was done using the null hypothesis of 0.5 and an AUC using the alternative hypothesis of 0.8 using a two-sided *z*-test at a significance level of 0.05. Therefore, further validation studies with more samples are needed for this model. Numeric analysis with a minimum sample size of 26 (13 each from the positive and negative groups) would be needed to achieve an adequate power (more than 0.8) to detect any significant difference.

Growing evidence has shown that peritoneal fluid microenvironment regulates endometrial cells derived from retrograde menstruation and superficial endometriosis (Koninckx *et al*., 1998). There is a concept that superficial endometriosis is regulated by peritoneal fluid, whereas deep endometriosis is regulated by factors in the bloodstream (Koninckx *et al*., 1998). Heilier *et al*. (2005) demonstrated that PCDDs/PCDFs, and dioxin-like PCDDs in serum burdens are associated with peritoneal endometriosis and deep endometriotic (adenomyotic) nodules. PCDDs/PCDFs levels in the serum of patients with deep endometriotic nodules were especially significantly increased compared with controls and peritoneal endometriosis (Heilier *et al*., 2005). In the present study, by laparoscopic examination, 9 of 10 patients (90%) were confirmed as having superficial endometriosis, and only one patient was diagnosed as having a deep adenomyoma, according to the criteria of Heilier *et al*. In this context, our results may not have a detection power to find a difference in the serum TEQ levels of PCDDs and PCDFs between the endometriosis patients and the controls (Table II). However, the incidence of both the higher levels of PCDDs and PCDFs in local ascites were significantly increased in the endometriosis patients. These results suggest that the PCDDs that escape to ascites, combining the higher levels of PCDFs, may play an important role in the pathogenesis of peritoneal endometriosis. On the other hand, there may still be another possibility, where the elevated concentration of PCDDs and PCDFs could have been the result from the modified metabolism of the peritoneal microenvironment affected by existing endometriosis.

The strengths of the present study include that we first established the best clinical diagnostic means for endometriosis because the patients and controls could be successfully diagnosed by laparoscopic examination alone, and we subsequently used a high-resolution gas chromatography/isotope-dilution mass spectrometry to determine

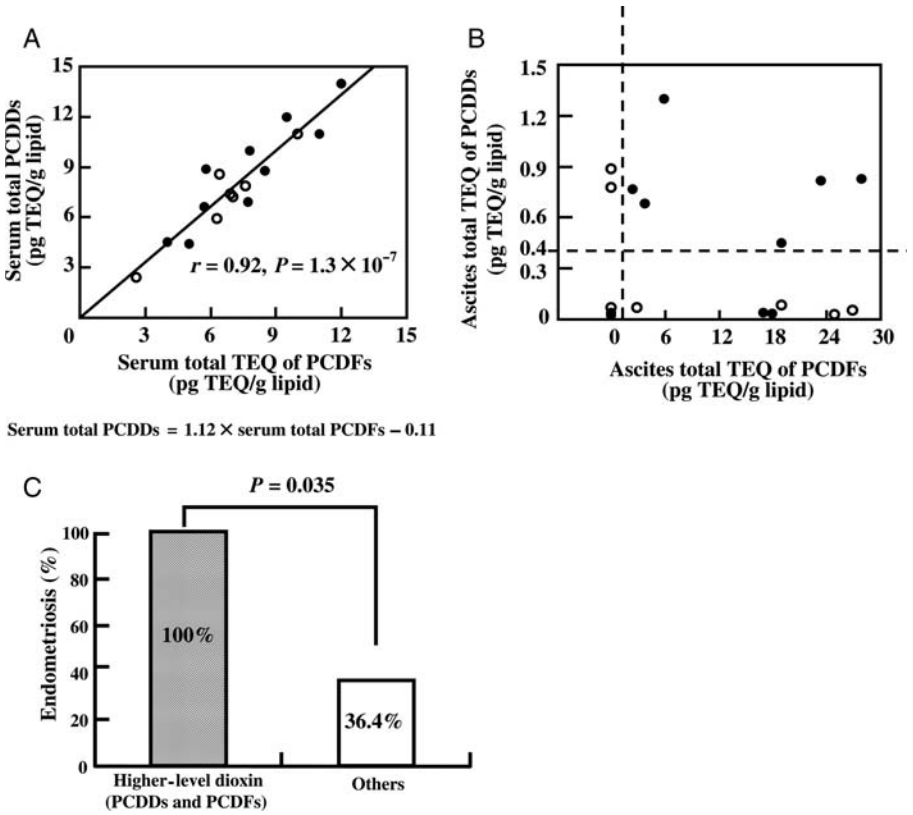

Figure 5 Distribution of analyzed cases in PCDD and PCDF values of serum and ascites. Co-relations were analyzed between PCDD and PCDF values in serum (**A**) ($r = 0.92$, $P < 0.001$) and ascites (**B**) ($r = 0.02$, $P = 0.93$) denoting endometriosis patients as closed circles with control as open circles. As noted in B, there is a significant association of simultaneous higher levels of PCDDs and PCDFs in ascites compared with endometriosis (**C**). According to values in ascites, the subjects were divided into two groups, the higher-level group (PCDDs >0.4 pg TEQ/pg lipid with detectable PCDFs TEQ levels) and the lower-lever group. The percentages of endometriosis are shown. Statistical analysis by the χ^2-test showed significant association with endometriosis ($P = 0.035$), where the OR was 2.5 (endometriosis versus controls; 95% CI: 1.17–5.34).

Table III Biological activity-dependent comparisons of top three PCBs between endometriosis and controls.

	Control ($n = 7$)	Endometriosis ($n = 10$)	*P*-value
Anti-estrogenic PCBs in ascites	16.4 ± 8.8	15.6 ± 4.6	0.82[a]
Estrogenic PCBs in ascites	11.2 ± 7.3	10.4 ± 3.9	0.79[a]
Anti-estrogenic PCBs in serum	6.3 ± 3.5	5.9 ± 1.7	0.63[b]
Estrogenic PCBs in serum	4.1 ± 2.7	3.6 ± 1.5	0.81[b]

Anti-estrogenic PCBs: PCB126, PCB118, PCB156; estrogenic PCBs: PCB126; data are presented as the mean ± SD (pg TEQ/g lipid);
[a]Student's *t*-test;
[b]Mann–Whitney *U*-test.

all 29 dioxin and dioxin-like compounds. We used infertile women as controls, since it is practically and ethically inconceivable to perform a diagnostic laparoscopy on normal healthy women as controls. Therefore, the references in this study as well as the present study itself should be read especially carefully while paying attention to the biases and restrictions of each. In addition, as another bias, the particular disease characteristics of endometriosis should be kept in mind. Even though the laparoscopic findings of endometriosis were negative, that alone would not necessarily guarantee that the subject would not develop endometriosis at some time in the future. This scope regarding disease character should be taken into account in all such reports.

To our knowledge, the present study is the first to demonstrate that PCDDs and PCDFs in ascites are linked with endometriosis. Therefore, further studies of larger epidemiological samples with complement populations as controls are warranted. Our results suggest that some dioxins in ascites likely play a role in the onset and exacerbation of endometriosis and reflect the changed intraperitoneal microenvironment by the coexisting tissues of endometriosis.

Authors' roles

L.Y.C. contributed all works including in study design, execution, analysis, manuscript drafting and critical discussion. S.I. supervised all works including in study design, execution, analysis, manuscript

drafting and critical discussion. T.S. contributed mainly in study execution, manuscript drafting and critical discussion. K.G. contributed mainly in study execution, manuscript drafting and critical discussion. E.N. contributed mainly in study execution, manuscript drafting and critical discussion. T.S. contributed mainly in study execution, manuscript drafting and critical discussion. H.K. contributed mainly in data analysis, manuscript drafting and critical discussion.

Acknowledgements

The authors thank Mr Robert E. Brandt, CEO, MedEd Japan, for editing the manuscript.

References

Akutsu K, Takatori S, Nozawa S, Yoshiike M, Nakazawa H, Hayakawa K, Makino T, Iwamoto T. Polybrominated diphenyl ethers in human serum and sperm quality. *Bull Environ Contam Toxicol* 2008;**80**:345–350.

Anger DL, Foster WG. The link between environmental toxicant exposure and endometriosis. *Front Biosci* 2008;**13**:1578–1593.

Arisawa K, Matsumura T, Tohyama C, Saito H, Satoh H, Nagai M, Morita M, Suzuki T. Fish intake, plasma omega-3 polyunsaturated fatty acids, and polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans and co-planar polychlorinated biphenyls in the blood of the Japanese population. *Int Arch Occup Environ Health* 2003;**76**:205–215.

Arisawa K, Takeda H, Mikasa H. Background exposure to PCDDs/PCDFs/PCBs and its potential health effects: a review of epidemiologic studies. *J Med Invest* 2005;**52**:10–21.

Balasch J, Creus M, Fábregues F, Carmona F, Ordi J, Martinez-Román S, Vanrell JA. Visible and non-visible endometriosis at laparoscopy in fertile and infertile women and in patients with chronic pelvic pain: a prospective study. *Hum Reprod* 1996;**11**:387–391.

Bofinger DP, Feng L, Chi LH, Love J, Stephen FD, Sutter TR, Osteen KG, Costich TG, Batt RE, Koury ST *et al.* Effect of TCDD exposure on CYP1A1 and CYP1B1 expression in explant cultures of human endometrium. *Toxicol Sci* 2001;**62**:299–314.

Bruner-Tran KL, Yeaman GR, Crispens MA, Igarashi TM, Osteen KG. Dioxin may promote inflammation-related development of endometriosis. *Fertil Steril* 2008;**89**:1287–1298.

Centor RM. Signal detectability: the use of ROC curves and their analyses. *Med Decis Making* 1991;**11**(2):102–106.

Cummings AM, Metcalf JL, Birnbaum L. Promotion of endometriosis by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats and mice: time-dose dependence and species comparison. *Toxicol Appl Pharmacol* 1996;**138**:131–139.

Donnez J, Van Langendonckt A, Casanas-Roux F, Van Gossum JP, Pirard C, Jadoul P, Squifflet J, Smets M. Current thinking on the pathogenesis of endometriosis. *Gynecol Obstet Invest* 2002;**54**:52–62.

Eskenazi B, Mocarelli P, Warner M, Samuels S, Vercellini P, Olive D, Needham LL, Patterson DG, Brambilla P, Gavoni N *et al.* Serum dioxin concentrations and endometriosis: a cohort study in Seveso, Italy. *Environ Health Perspect* 2002;**110**:629–634.

Foster WG. Endocrine toxicants including 2,3,7,8-terachlorodibenzo-p-dioxin (TCDD) and dioxin-like chemicals and endometriosis: is there a link? *J Toxicol Environ Health B Crit Rev* 2008;**11**:177–187.

Guo SW. The link between exposure to dioxin and endometriosis: a critical reappraisal of primate data. *Gynecol Obstet Invest* 2004;**57**:157–173.

Heilier JF, Nackers F, Verougstraete V, Tonglet R, Lison D, Donnez J. Increased dioxin-like compounds in the serum of women with peritoneal endometriosis and deep endometriotic (adenomyotic) nodules. *Fertil Steril* 2005;**84**:305–312.

Igarashi TM, Bruner-Tran KL, Yeaman GR, Lessey BA, Edwards DP, Eisenberg E, Osteen KG. Reduced expression of progesterone receptor-B in the endometrium of women with endometriosis and in cocultures of endometrial cells exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Fertil Steril* 2005;**84**:67–74.

Johnson KL, Cummings AM, Birnbaum LS. Promotion of endometriosis in mice by polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls. *Environ Health Perspect* 1997;**105**:750–755.

Klinge CM, Bowers JL, Kulakosky PC, Kamboj KK, Swanson HI. The aryl hydrocarbon receptor (AHR)/AHR nuclear translocator (ARNT) heterodimer interacts with naturally occurring estrogen response elements. *Mol Cell Endocrinol* 1999;**157**:105–119.

Koninckx PR, Kennedy SH, Barlow DH. Endometriotic disease: the role of peritoneal fluid. *Hum Reprod Update* 1998;**4**:741–751.

LaKind JS. Recent global trends and physiologic origins of dioxins and furans in human milk. *J Expo Sci Environ Epidemiol* 2007;**17**:510–524.

Louis GM, Weiner JM, Whitcomb BW, Sperrazza R, Schisterman EF, Lobdell DT, Crickard K, Greizerstein H, Kostyniak PJ. Environmental PCB exposure and risk of endometriosis. *Hum Reprod* 2005;**20**:279–285.

Mayani A, Barel S, Soback S, Almagor M. Dioxin concentrations in women with endometriosis. *Hum Reprod* 1997;**12**:373–375.

Niskar AS, Needham LL, Rubin C, Turner WE, Martin CA, Patterson DG Jr, Hasty L, Wong LY, Marcus M. Serum dioxins, polychlorinated biphenyls, and endometriosis: a case–control study in Atlanta. *Chemosphere* 2009;**74**:944–949.

Ohtake F, Takeyama K, Matsumoto T, Kitagawa H, Yamamoto Y, Nohara K, Tohyama C, Krust A, Mimura J, Chambon P *et al.* Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. *Nature* 2003;**423**:545–550.

Patterson DG Jr, Hampton L, Lapeza CR Jr, Belser WT, Green V, Alexander L, Needham LL. High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Anal Chem* 1987;**59**:2000–2005.

Patterson DG Jr, Turner WE, Isaacs SG, Alexander LR. A method performance evaluation and lessons learned after anlayzing more than 5000 human adipose tissue, serum, and breast milk samples for polychlorinated dibenzo-p-dioxins (PCDFs) PCDDs and dibenzofurans (PCDFs). *Chemosphere* 1990;**20**:829–836.

Pauwels A, Schepens PJ, D'Hooghe T, Delbeke L, Dhont M, Brouwer A, Weyler J. The risk of endometriosis and exposure to dioxins and polychlorinated biphenyls: a case–control study of infertile women. *Human Reprod* 2001;**16**:2050–2055.

Porpora MG, Ingelido AM, di Domenico A, Ferro A, Crobu M, Pallante D, Cardelli M, Cosmi EV, De Felip E. Increased levels of polychlorobiphenyls in Italian women with endometriosis. *Chemosphere* 2006;**63**:1361–1367.

Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril* 1997;**67**:817–821.

Rier SE. Environmental immune disruption: a comorbidity factor for reproduction? *Fertil Steril* 2008;**89**:103–108.

Rier S, Foster WG. Environmental dioxins and endometriosis. *Toxicol Sci* 2002;**70**:161–170.

Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Fundam Appl Toxicol* 1993;**21**:433–441.

Rier SE, Turner WE, Martin DC, Morris R, Lucier GW, Clark GC. Serum levels of TCDD and dioxin-like chemicals in Rhesus monkeys chronically

exposed to dioxin: correlation of increased serum PCB levels with endometriosis. *Toxicol Sci* 2001;**59**:147–159.

Safe S, Wang F, Porter W, Duan R, McDougal A. Ah receptor agonists as endocrine disruptors: antiestrogenic activity and mechanisms. *Toxicol Lett* 1998;**102–103**:343–347.

Strathy JH, Molgaard CA, Coulman CB, Melton LJ 3rd. Endometriosis and infertility: a laparoscopic study of endometriosis among fertile and infertile women. *Fertil Steril* 1982;**38**:667–672.

Tariverdian N, Theoharides TC, Siedentopf F, Gutiérrez G, Jeschke U, Rabinovich GA, Blois SM, Arck PC. Neuroendocrine-immune disequilibrium and endometriosis: an interdisciplinary approach. *Semin Immunopathol* 2007;**29**:193–210.

Tsukino H, Hanaoka T, Sasaki H, Motoyama H, Hiroshima M, Tanaka T, Kabuto M, Niskar AS, Rubin C, Patterson DG *et al*. Associations between serum levels of selected organochlorine compounds and endometriosis in infertile Japanese women. *Environ Res* 2005; **99**:118–125.

Tsutsumi O, Uechi H, Sone H, Yonemoto J, Takai Y, Momoeda M, Tohyama C, Hashimoto S, Morita M, Taketani Y. Presence of dioxins in human follicular fluid: their possible stage-specific action on the development of preimplantation mouse embryos. *Biochem Biophys Res Commun* 1998;**250**:498–501.

Van den Berg M, Birnbaum L, Bosveld AT, Brunström B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW *et al.* Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 1998;**106**:775–792.

Yu J, Wang Y, Zhou WH, Wang L, He YY, Li DJ. Combination of estrogen and dioxin is involved in the pathogenesis of endometriosis by promoting chemokine secretion and invasion of endometrial stromal cells. *Hum Reprod* 2008;**23**:1614–1626.