Associations of paternal serum dioxin-like polychlorinated biphenyl concentrations with IVF success: A pilot study

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ABSTRACT

Dioxin-like polychlorinated biphenyls (DL-PCBs) are environmental pollutants that have been associated with impaired semen quality. However, research on the potential impact of paternal exposure to DL-PCBs and the risk of adverse pregnancy outcomes are limited. We examine the relationship between serum DL-PCB concentrations and IVF outcomes among 42 males seeking fertility treatment. Concentrations of 12 serum DL-PCBs were analyzed by high-resolution gas chromatography coupled to high-resolution mass spectrometry. Modified Poisson regressions, adjusted for confounders, were used to assess bivariate associations and to estimate risk ratios (RRs) between DL-PCBs and binary IVF outcomes. The median concentration (25th-75th percentiles) of the sum of the 12 DL-PCBs (∑DL-PCBs) obtained for the patients was 5.42 (3.78–7.78) ng/g lipid. No statistically significant association between DL-PCB levels and embryo quality was found. However, men with high serum PCB-77 concentrations present more probability of high-quality embryos (RR: 0.292; 95% CI: 0.090–0.942), whereas the opposite trend is observed for men with lower serum levels of PCB-156 (RR: 7.960; 95% CI: 1.020–62.100), who present increased odds of high-quality embryos. Serum concentrations of PCB-126 and PCB-114 were associated with decreased implantation rates (p < 0.05). Moreover, PCB-77 and ∑non-ortho PCBs were significantly associated with a lower likelihood of clinical pregnancy (p < 0.05). A lower likelihood of live birth was associated with higher levels of PCB-77, PCB-105, PCB-118, and recording significant differences for ∑non-ortho PCBs, ∑mono-ortho PCBs, and ∑DL-PCBs (p < 0.05).

These findings suggest that paternal DL-PCB exposure before conception may be related to pregnancy endpoints. However, DL-PCB measurement were limited to male partners. Therefore, we propose that future studies with larger population sizes should include both maternal and paternal factors.

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IVF
Embryo quality
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Clinical pregnancy
Live birth

1. Introduction

Environmental pollutants exert adverse physiological effects on testicular function and male fertility (Swan, 2006). Among others, highly toxic persistent organic pollutants including dioxin-like compounds such as dioxin-like polychlorinated biphenyls (DL-PCBs) negatively impact the human reproductive system (Leijs et al., 2014). Although emissions of these compounds have decreased during recent years, the general population is still exposed to detectable levels (Costopoulou et al., 2006; Zubero et al., 2011; De Felip et al., 2014; Domingo et al., 2017; Brajenović et al., 2018). Similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), one of the most toxic pollutants known, DL-PCBs bind to the aryl hydrocarbon receptor (AhR) (van den Berg et al., 2006), showing a decrease in sperm quality and sperm DNA integrity (Meeker and Hauser, 2010). Further, they are identified as endocrine-disrupting compounds (EDCs), disrupting hormonal functions, especially during early development and pregnancy (Mitro et al., 2015).
Early reports show evidence on the association between dioxin/PCB exposure and semen quality (Guo et al., 2000; Hsu et al., 2003; Dhogo et al., 2006; Toft et al., 2007; Mocarelli et al., 2011; Mínguez-Alarcón et al., 2016). However, the potential impact of paternal exposure to environmental chemicals in general, and dioxin-like compounds in particular, and the risk of adverse pregnancy effects have been correctly identified in a smaller and delineated set of research (Cordier, 2008). Instead, reports on mice for example, accurately demonstrated that adult males exposed in utero to TCDD displayed diminished fertility and an increased risk of spontaneous preterm birth when compared to their unexposed mating partners (Ding et al., 2011; McConaha et al., 2011; Bruner-Tran et al., 2014).

Other studies have shown that utero/developmental TCDD exposure in rats, decreases sperm quality in all adult male rat offspring (F1, F2, F3 generations) at least at one dose, and this effect persists into the next generations (Sanabria et al., 2016). Other reports show that DL-PCB congeners 77 and 126 decreased serum testosterone concentrations in adult male rats exposed in utero (Faqui et al., 1996). In addition, a study carried out by Hsu et al. (2004) indicated that postnatal exposure to individual PCB-77 was associated with impairment of sperm functions and fertilization ability in mature rats. Furthermore, there is epidemiological evidence for potentially adverse outcomes after paternal exposure to DL-PCBs. For instance, epidemiological studies have associated the effects of dioxin or DL-PCB exposure and adverse effects on pregnancy outcomes, such as miscarriages (Donovan et al., 1984), altered sex ratio of children (Ryan et al., 2002) and congenital malformations (Dimich-Ward et al., 1996; Ngo et al., 2010). Additionally, Buck Louis et al. (2013) showed that male exposure to DL-PCBs, such as non-ortho PCBs 156 and 157, are associated with a longer time-to-pregnancy (TTP) and an increased risk of infertility.

Currently, epidemiology derived evidence from the use of assisted reproductive technology (ART) supports the crucial contribution of the male gamete during early embryo development (Barroso et al., 2009; Stuppa et al., 2015). Recently we reported that serum DL-PCB concentrations largely affect semen quality among males seeking fertility treatments (Paul et al., 2017). Based on our earlier results, here we hypothesized that greater paternal serum DL-PCB levels would be associated with poorer embryo quality and a lower likelihood of pregnancy and live birth. Therefore, studying couples undergoing in vitro fertilization (IVF) provides us with the opportunity to explore the impact of paternal preconception serum concentrations of DL-PCBs on pregnancy endpoints.

2. Materials and methods

2.1. Study design and subject selection

The current study was conducted as part of a cross-sectional study on DL-PCB exposure and semen quality (see Paul et al., 2017 for details). The original population included 56 couples seeking IVF treatment between May 2012 and June 2014 at the IVF Spain clinic (Alicante, Spain), whose males (30–55 age range) had DL-PCB measurements in serum. Patrons with known factors related to male infertility such as varicocele, post-vasectomy or cryptorchidism, endocrine hypogonadism (abnormal hormonal concentrations), immune infertility, genetic disease, infection, anomalies in the karyotype or Y chromosome microdeletions were excluded from the study (6 out of 56 subjects, resulting in a final n = 50).

Since the present study assesses the influence of paternal DL-PCB exposure on embryo development, the following inclusion criteria were subsequently applied to the cohort:

- Couples whose males had DL-PCB measurements.
- Couples who underwent only one fresh IVF cycle.
- Young female partners (<37 years old).
- Women not affected by known infertility factors such as polycystic ovarian syndrome (PCOS), endometriosis or showing diminished ovarian reserve or low response to ovarian stimulation.
- Female patients treated with ovarian follicle stimulation using short GnRH antagonist protocol.
- Couples with body mass index (BMI) ≤ 30 kg/m².
- Only non-smokers.
- Couples not using donor oocytes or sperm.

The criteria reported above were applied in order to avoid bias with factors that might affect IVF outcomes (van Loendersloot et al., 2010; Ferraretti et al., 2011; Stimpfel et al., 2015; Lucovnik et al., 2018).

Fig. 1 shows the flow chart of inclusion and drop out of patients included in the study. Among 56 eligible patients asked to participate, 14 couples were ultimately excluded. Hence, the study group analyzed here comprises a subsample of 42 couples from our previous report (Paul et al., 2017).

A complete clinical examination was performed on every patient, and a questionnaire was used to collect individual information, including relevant data on demographics, medical history, lifestyle factors and questions about potential occupational exposures to environmental chemical compounds. All participants provided informed consent after a research nurse explained the study procedures and all questions were answered. A written consent form of each subject was taken after explaining the aims and objectives of the study and its benefits to individuals and society. The Human Research Committee of the University General Hospital of Alicante (Spain) approved this study, in accordance with the principles of the Declaration of Helsinki.

2.2. Clinical procedures

Couples were selected at their first IVF treatment cycle (conventional in vitro fertilization, IVF; or Intracytoplasmic sperm injection, ICSI). Trained research nurses obtained all IVF outcome variables from the medical record. Each couple contributed only one treatment cycle to the study. To avoid potential confounding by treatment protocol, all female patients were treated with ovarian follicle stimulation using short GnRH antagonist protocol with rFSH/hMG after completing a cycle of oral contraceptives.

Recombinant FSH (Gonal-F) followed by hMG (Menopur) was used starting on cycle day 3 of the menstrual cycle. When the lead follicle reached 14 mm in diameter, 0.25 mg cetrorelix (Cetrotide; Serono) were added until the day of hCG (Ovitrelle) administration. Serum estradiol measurements and transvaginal ultrasonography were employed to monitor follicular maturation and endometrial development.

Ovarian stimulation was induced with 10,000 IU of serum beta-human chorionic gonadotropin (β-hCG), when at least three dominant follicles ≥17 mm diameter were noted and peak estradiol level was higher than 600 pg/mL. Oocyte retrieval was performed approximately 36 h.

The endpoints assessed included intermediate IVF outcomes (embryo quality) and clinical IVF outcomes (implantation, clinical pregnancy and live birth) based on previous reports (ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2017).

2.2.1. Embryo quality

Embryo quality was described using criteria based on blastomeres size and number, the degree of fragmentation, and the presence of multinucleated blastomeres. We dichotomized embryo quality as high-quality versus low-quality embryo. Embryos with an absence of multinucleated blastomeres, four or five blastomeres on day 2, seven or more cells on day 3, and ≤20% anucleated fragments were regarded as high-quality embryos (van Rooyen et al., 1999). Embryos were transferred on the second or third day after fertilization. In 3 cases, embryos were not transferred due to impaired quality.

2.2.2. Implantation

Determination of serum β-hCG is an important tool for the diagnosis of implantation outcome of in vitro fertilization cycles (Bjercke et al.,
Implantation was defined as β-hCG level $> 5$ mIU/mL, typically measured 17 days after embryo transfer. When levels did not reach 5 mIU/mL, implantations were considered negative (failure of implantation).

2.2.3. Clinical pregnancy

The presence of a gestational sac on ultrasound and a fetal heartbeat at 7 weeks amenorrhea was considered a clinical pregnancy.

2.2.4. Live birth

Live birth was defined as an infant born alive after 24 weeks’ gestation.

2.3. Serum DL-PCB analysis

Serum samples were collected from male partners on the same day as semen samples obtained, based on Paul et al. (2017), before the day of oocyte retrieval. Samples were immediately stored in the dark at $-20^\circ$C until DL-PCB analysis. We determined 12 coplanar DL-PCB congeners, comprising 4 non-ortho-substituted PCBs (77, 81, 126 and 169) and 8 mono-ortho-substituted PCBs (105, 114, 118, 123, 156, 157, 167 and 189), using a recently developed method for the analysis of DL-PCBs in serum (Molto et al., 2016). Serum samples were analyzed using high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC-HRMS).

Concentrations of PCBs were adjusted for total serum lipids. DL-PCB concentrations are reported both individually and as the sum of all congeners assayed ($\sum$DL-PCBs) and expressed in nanograms of compound per gram of total lipids (ng/g lipid). Serum total cholesterol and triglycerides were measured enzymatically, and total lipids concentrations calculated by Phillips formula (Phillips et al., 1989; Bernert et al., 2007).

Because of financial constraints and the complexity of the analysis, it was not possible to measure PCBs in serum from female partners in the study.

2.4. Statistical analyses

We explored the relationship between IVF outcomes and DL-PCB congeners. Age (years) and body mass index (BMI; kg/m$^2$) were considered in both the male patient and his female partner (Sharma et al., 2013) (Table 1). We followed statistical methods previously outlined (Bloom et al., 2017; Dodge et al., 2020). The Shapiro-Wilk test was first performed in order to test the distribution and equal variance in DL-PCB values, showing that 86.6% of the PCBs were not normally distributed ($W = 0.49$ to 0.93; $p < 0.05$). In order to discard a possible selection bias, the non-parametric Mann–Whitney $U$ test was used to compare DL-PCB concentrations, seminal and demographic (age and BMI) parameters between couples included in our analyses and patients excluded (see Fig. 1) showing non-significant differences. We then used modified Poisson regression model with robust variance estimation, to estimate the Relative Risk (RR) and corresponding 95% confidence interval (CI) between log-transformed DL-PCB concentrations and dichotomous IVF outcomes, which included embryo quality (modeled as the proportion of high/low quality embryos), the successes of embryo implantation, clinical pregnancy and live birth, with adjustment for age, BMI and...
serum lipids confounders. We choose a modified Poisson regression approach due to our modeling of dichotomous outcomes and to prevent the instability and convergence given the sample size (Dodge et al., 2020). Further, the RR is generally the preferred measure of association (Dodge et al., 2020). Finally, correlations between serum DL-PCB concentrations were assessed using Spearman (ρ) correlation coefficients. Descriptive and statistical analyses were performed using IBM® SPSS® Statistics 22.0 (IBM, Armonk, NY, USA) and Jamovi 1.2.27. Results are presented as median and 25th-75th percentiles for each DL-PCB congener. Two-sided p-values <0.05 were considered to be statistically significant.

3. Results

3.1. Characteristics of patients

The demographic characteristics and infertility diagnoses are shown in Table 1. At the time of the procedure, subjects’ age (years) was 38.53 ± 5.11 for males and 32.97 ± 2.10 for females, respectively. Regarding body weight, mean BMI (kg/m²) for males and females were 24.50 ± 2.46 and 23.13 ± 1.78, respectively, being 76.19% of males and 85.71% of females classified as normal in weight (18.5 ≤ BMI ≤24.9, in kg/m²). Most couples had no previous IVF attempts (71%) and all participants were white and nonsmokers. Regarding infertility diagnoses among couples, in 48% of them a male factor infertility was diagnosed, 31% presented tubal factor infertility (female infertility), and 21% had unexplained infertility.

Semen parameters and IVF outcomes are reported in Table 2. From the 42 couples selected for the study, 27 underwent ICSI treatment, whereas 15 were subjected to a conventional IVF. A total of 631 oocytes were retrieved, yielding an average (mean ± standard deviation, per couple) of 15.03 ± 5.46 oocytes per couple, 605 (14.40 ± 5.42) were mature (MII) and 430 (10.23 ± 3.98) of them were fertilized. Three days later, embryos were assessed for embryo quality, of which 211 (5.03 ± 0.22) were classified as high quality. The majority of embryos (64%) were transferred on day 3. There were 23 (55%) positive embryo implantations, 22 (52%) clinical pregnancies and 15 (36%) live births.

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N (%)</th>
<th>Mean ± σ</th>
<th>Median</th>
<th>Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42 (100%)</td>
<td>38.53 ± 5.11</td>
<td>39</td>
<td>30-46</td>
</tr>
<tr>
<td>Female</td>
<td>42 (100%)</td>
<td>32.97 ± 2.10</td>
<td>33</td>
<td>28-37</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42 (100%)</td>
<td>24.50 ± 2.46</td>
<td>24.18</td>
<td>17.97-31.25</td>
</tr>
<tr>
<td>Female</td>
<td>42 (100%)</td>
<td>23.13 ± 1.78</td>
<td>23.12</td>
<td>19.37-26.25</td>
</tr>
<tr>
<td>Smoker</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male nonsmoker</td>
<td>42 (100%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Female nonsmoker</td>
<td>42 (100%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>White race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42 (100%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Female</td>
<td>42 (100%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Infertility diagnoses</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male factor alone</td>
<td>42 (100%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Female factor alone</td>
<td>13 (31%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unexplained</td>
<td>9 (21%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are presented as N (%) or mean. σ = standard deviation. Min-Max = minimum and maximum values.

BMI = body mass index.

Table 2

<table>
<thead>
<tr>
<th>Semen Parameters</th>
<th>N (%)</th>
<th>Mean ± σ</th>
<th>Median</th>
<th>Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume (mL)</td>
<td>42 (100%)</td>
<td>2.74 ± 1.27</td>
<td>2.80</td>
<td>0.30-5.00</td>
</tr>
<tr>
<td>Sperm concentration (x10⁹/mL)</td>
<td>42 (100%)</td>
<td>45.07 ± 50.77</td>
<td>32.00</td>
<td>1.30-180.00</td>
</tr>
<tr>
<td>Sperm progressive motility (%)</td>
<td>42 (100%)</td>
<td>48.40 ± 30.39</td>
<td>60.00</td>
<td>2.00-90.00</td>
</tr>
<tr>
<td>Sperm viability (%)</td>
<td>42 (100%)</td>
<td>75.52 ± 20.81</td>
<td>85.00</td>
<td>15.00-95.00</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>42 (100%)</td>
<td>9.96 ± 5.92</td>
<td>10.00</td>
<td>1.00-23.00</td>
</tr>
<tr>
<td>IVF outcomes</td>
<td>N (%)</td>
<td>Mean ± σ</td>
<td>Median</td>
<td>Min-Max</td>
</tr>
<tr>
<td>IVF/ICSI attempts 0</td>
<td>30 (71.4%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>4 (9.5%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>≥2</td>
<td>8 (19%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Day 3 FSH (IU/L)</td>
<td>–</td>
<td>6.73 ± 1.72</td>
<td>6.50</td>
<td>3.00-13.00</td>
</tr>
<tr>
<td>Day 3 Estradiol (pg/mL)</td>
<td>–</td>
<td>2148 ± 1213</td>
<td>2069</td>
<td>285-6259</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>–</td>
<td>10.20 ± 1.80</td>
<td>9.70</td>
<td>5.00-12.00</td>
</tr>
<tr>
<td>ICSE</td>
<td>27 (64%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Retrieved oocytes</td>
<td>631 (100%)</td>
<td>15.03 ± 5.46</td>
<td>15.00</td>
<td>1.00-31.00</td>
</tr>
<tr>
<td>Mature oocytes MII</td>
<td>605 (96%)</td>
<td>14.40 ± 5.42</td>
<td>14.00</td>
<td>1.00-31.00</td>
</tr>
<tr>
<td>2 PN-zygotes c</td>
<td>430 (68%)</td>
<td>10.23 ± 3.98</td>
<td>10.00</td>
<td>1.00-20.00</td>
</tr>
<tr>
<td>Fertilization rate (%) c</td>
<td>–</td>
<td>74.08 ± 18.33</td>
<td>72.77</td>
<td>62.08-100</td>
</tr>
<tr>
<td>High quality embryos at day 3 c</td>
<td>211 (33%)</td>
<td>5.03 ± 0.22</td>
<td>0.48</td>
<td>0.09-1.00</td>
</tr>
<tr>
<td>Day of embryo transfer 2</td>
<td>15 (35.7%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>27 (64.3%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Number of embryos transferred 0</td>
<td>3 (7.1%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>13 (31.0%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>25 (59.5%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>1 (2.4%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Implantation</td>
<td>23 (55%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>22 (52%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Live birth</td>
<td>15 (36%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are presented as N (%) or mean. σ = standard deviation. Min-Max = minimum and maximum values.

IVF = in vitro fertilization. ICSI = Intracytoplasmic sperm injection. FSH = follicle stimulating hormone.

Implantation = β-hCG level ≥ 5 mIU/mL 17 days after embryo transfer.

Clinical pregnancy = presence of a gestational sac on ultrasound and a fetal heartbeat at 7 weeks amenorrhea.

Live birth = an infant born alive after 24 weeks of gestation.

Number of zygotes with 2 pronuclei. c Number of zygotes with 2 pronuclei/number of mature metaphase II oocytes.

van Roeyen et al., 1999.

3.2. Levels of DL-PCBs in serum

Descriptive statistics of the concentrations of DL-PCBs expressed as ng/g lipid in the serum samples are summarized in Table 3. The total levels of DL-PCBs (ΣΣDL-PCBs) obtained for the patients ranged from 2.02 to 13.23 ng/g lipid, with a median value of 5.42 ng/g lipid. The predominant congeners, in descending order, were PCBs 118, 156, and 105, with median values of 2.13, 0.87, and 0.45 ng/g lipid, respectively.

3.2.1. Correlations between individual PCBs

Considering that PCBs are found as mixtures and tend to be highly...
intercorrelated, Spearman (ρ) correlation coefficients were used to assess relationships between PCB congeners (Table S2). We decided to focus on those congeners associated with IVF outcomes in the present work, i.e., PCB-77, 105, 114, 118, 126 and 189.

Non-ortho PCB-77 is highly correlated with ∑non-ortho PCBs (ρ = 0.747; p < 0.001). In addition, PCB-77 also showed strong correlations with PCB-105 (ρ = 0.545; p < 0.01). Mono-ortho congener 105, presented strong correlations with PCB-118, non-ortho PCBs, mono-ortho PCBs and ∑DL-PCBs (ρ = 0.607–0.902; p < 0.001), being also significantly correlated with PCB-123 (ρ = 0.633; p < 0.01). With respect to PCB-114, we found a significant correlation between the congener and PCB-118, ∑mono-ortho and ∑DL-PCBs (ρ = 0.472–0.554; p < 0.01). PCB-118 was highly correlated with ∑mono-ortho PCBs and ∑DL-PCBs (ρ = 0.942–0.949; p < 0.001). Furthermore, high correlations were observed between PCB-118 and PCB-123 (ρ = 0.551; p < 0.01) and 156 (ρ = 0.504; p < 0.01). In addition, a strong correlation was observed between PCBs 126 and 169 (ρ = 0.519; p < 0.01). Finally, PCB-189 showed a high correlation with PCB-156 (ρ = 0.491; p < 0.01).

### 3.3. Clinical pregnancy

With respect to clinical pregnancy (Table 6), we found a significant association between lower likelihood of clinical pregnancy and higher levels of PCB-77 (RR: 0.275; 95% CI: 0.102–0.744; p = 0.022) and the sum of non-ortho PCBs (RR: 0.129; 95% CI: 0.023–0.715; p = 0.029).

### 3.3.4. Live birth

As can be seen in Table 7, we found statistically significant associations after adjustment for confounders, between high levels of ∑DL-PCBs (RR: 0.046; 95% CI: 0.004–0.431; p = 0.028) and a lower likelihood of live birth, also recording significant differences for ∑non-ortho PCBs (RR: 0.041; 95% CI: 0.008–0.192; p = 0.017) and ∑mono-ortho PCBs (RR: 0.073; 95% CI: 0.008–0.674; p = 0.045). By individual congeners, non-ortho PCB-77 (RR: 0.099; 95% CI: 0.038–0.256; p = 0.013), PCB-105 (RR: 0.107; 95% CI: 0.024–0.475; p = 0.019), and PCB-118 (RR: 0.225; 95% CI: 0.088–0.572; p = 0.046) were associated with lower odds of live birth. Although not statistically significant, PCB-114 (RR: 0.192; 95% CI: 0.045–0.822) and PCB-123 (RR: 0.203; 95% CI: 0.063–0.652) were also associated with lower chances of live birth.

### 4. Discussion

When applying modified Poisson regression models, inverse significant associations after adjustment for confounders were found between paternal serum PCBs and clinical IVF outcomes. We detected associations between higher serum concentrations of PCBs 126 and 114 and reduced likelihood of embryo implantation. Interestingly, higher levels of non-ortho PCB-77 were associated with lower likelihood of clinical pregnancy and live birth. We also detected significantly lower probabilities of live birth among couples whose male partners presented higher serum concentrations of PCBs 105 and 118, as well as ∑non-ortho PCBs, ∑mono-ortho PCBs and ∑DL-PCBs.
The mean of total sum of DL-PCBs ($\sum$DL-PCBs) obtained for the patients in our study was 5.80 ng/g lipid (see Table 3). Comparing with previous studies carried out in general population not directly exposed to recognized emission sources, the level found is lower than the level in serum from the general population of Korea (10.30 ng/g lipid) (Park et al., 2009), and in the same order of the mean concentration found in serum from the general Chinese population (3.48 and 2.82 ng/g lipid, in 2011 and 2017 respectively) (Lin et al., 2018).

Several authors have studied the DL-PCBs in blood serum samples from populations living in industrial areas or near urban waste treatment or incineration plants. Park et al. (2009) found a total DL-PCB level of 22.45 ng/g lipid in the blood from people living near a municipal solid waste incinerator in Korea. Zubero et al. (2017), found a total DL-PCB concentration value of 7.41 ng/g lipid in serum samples from residents living near a municipal waste incinerator in Bilbao, Spain. The values obtained for the male patients in our study are lower than the level in serum from the general Chinese population (3.48 and 2.82 ng/g lipid, in 2011 and 2017 respectively) (Lin et al., 2018).
lower than those presented by these studies.

We also contrasted our DL-PCB levels with the results from the concentrations reported in the U.S National Health and Nutrition Examination Survey (NHANES) for the period 2005–2016 (CDC, 2021). The mean serum levels of non-ortho DL-PCBs in the current study were higher than those levels in US non-hispanic white males aged 40–59, with values in the range of 1.7–19.9 pg/g lipid (CDC, 2021). Nevertheless, the median values of each mono-ortho PCB were lower in serum of males of our study compared with the NHANES data, with the exception of PCB-123 for which the U.S Survey does not report a value because the proportion of results below limit of detection was too high to provide a valid result.

In agreement with our data, several animal studies have reported a significant decrease in IVF success rates at low and environmental level doses of mixtures of PCBs (Campagna et al., 2002; Kholkute et al., 1994). Moreover, epidemiological research, which have evaluated the long-term health consequences of exposure to TCDD among Vietnam War veterans and their offspring (Ngo et al., 2006, 2010), concluded that clear evidence exists for an association between dioxin exposure and human congenital disorders. Instead, studies on the effects of occupational exposure to TCDD indicate that the increases in the risk of spontaneous abortion (Schmitt et al., 2014). Additionally, recent data demonstrate that male factors such as sperm motility, viability, and sperm morphology (Paul et al., 2017).

More recently, the Longitudinal Investigation of Fertility and the Environment (LIFE) Study reported male serum concentration of 36 PCBs (Buck Louis et al., 2013). Comparing these results to our findings remains difficult, since the LIFE Study analyzed the relationship between PCBs and couple fecundity measured by TTP. For instance, they found that serum concentrations of DL-PCBs 118, 156, 157, and 167 in the male partners were inversely associated with couples’ fecundity. Interestingly, PCBs in male partners were more often associated with decreased fecundity than their female partners. Increased TTP reflects unobserved intermediate and clinical endpoints such as failed fertilization, altered embryo development, or implantation failure. Even though outcomes following a single IVF cycle were measured in the present study and not TTP, our findings point in the same direction as the decrease of fecundity reported in the LIFE study.

These findings underscore the critical role of paternal exposure to environmental chemicals in reproductive outcomes. In turn, these adverse reproductive effects may result from the endocrine disrupting activities of the environmental chemicals (Sharpe and Irvine, 2004). DL-PCBs are often regarded as antiestrogenic due to their ability to act through AhR (Plikova et al., 2005). In this context, we previously found that mono-ortho PCB-189 was negatively correlated with male factors such as sperm motility, viability, and sperm morphology (Paul et al., 2017).

The mechanism by which paternal exposure before conception may affect the development of the embryo is still unclear. Cumulative evidence suggests that in addition to genome sperm, various epigenetic factors such as DNA methylation, histone modifications and spermatozoal RNAs (Carrell, 2013; Soubry et al., 2014) may play an essential role in successful fertilization and early development of the embryo (Zajitnitschek et al., 2014). Additionally, recent data demonstrate that male dioxin exposure during the preconception period induces epigenetic modifications in male gametogenesis and abnormal development of the offspring (Estill and Krawetz, 2016; Pilsner et al., 2017, 2018), resulting in offspring with impaired fertility and preconception outcomes. Future studies about the influence of preconception paternal exposure to dioxin-like compounds and epigenetic changes should be considered.

With regard to the Spearman correlation analysis (see Table S2), it revealed that PCB-77 was highly intercorrelated with Σnon-ortho PCBs. We also observed that PCB-118, Σnon-ortho PCBs, Σmono-ortho PCBs and ΣDL-PCBs showed a very high correlation with PCB-105. PCB-114 showed a weak correlation, but still significant, with PCB-126, one of the most toxic congeners (Bhavsar et al., 2008). PCB-118 was highly correlated with Σmono-ortho PCBs and ΣDL-PCBs. Besides, the highly toxic non-ortho PCBs 126 and 169 were strongly correlated.

The high degree of correlation observed with the highly chlorinated PCB 118, 156 and 105 is of special interest because they were found to be the most abundant in serum in the studied group. Former studies have reported these highly chlorinated congeners as markers for occupational (Seegal et al., 2011) and background (dietary) exposure (Nøstbakken et al., 2021). These data suggest that a possible source of exposure to DL-PCBs in our study group might be through diet and/or occupational exposure.

Due to the number of IVF couples analyzed, our results should be considered preliminary and examined in a larger cohort. For example, we had limited power to assess important modifying clinical factors such as the stimulation protocol used and the fertilization procedure. Besides, despite experimental and epidemiologic evidence of an association between diminished fecundity and exposure in females, our cohort was limited to male partners. Previous studies examining the relationship between maternal exposure to PCBs and IVF outcomes have demonstrated adverse outcomes, including oocyte maturity, oocytes fertilization, embryo quality and implantation failure. One such study (Mahalingaiah et al., 2012) was carried out on a prospective cohort study of 766 women (who underwent 827 IVF cycles). The study assessed the association between serum levels of each congener or group of them (PCBs grouped by biological activity and total of PCBs) with implantation failure, chemical pregnancy, and spontaneous abortion. The researchers found a significant trend for serum levels of total PCBs and the odds of implantation failure. Nonetheless, no association was observed for other congeners or congener groupings with different IVF outcomes (Mahalingaiah et al., 2012). Another investigation reported higher serum levels of PCB-153 (46.2 ng/g lipid) and the sum of all measured PCB congeners (ΣPCBs) associated with significantly elevated dose-dependent odds of failed implantation among IVF patients in Boston, MA (Meeker and Hauser, 2010).

Bloom et al. (2017) reported associations between individual ovarian follicular fluid (FF) PCBs and IVF endpoints among women undergoing infertility treatment. The authors found a stronger association between FF PCB-105 levels and lower oocyte fertilization. Moreover, FF levels of PCBs 87, 149, the sum of two PCB congeners with anti-estrogenic activity (PCBs 105 and 118), and total PCBs were associated with poorer embryo quality. Besides, there was a statistically significant association of implantation failure with FF levels of PCBs 28, 66, 146, and congener groupings (estrogenic PCBs and total PCBs). In Bloom’s study (2017), reduction of odds of live birth was associated with levels of individual PCBs 66, 74, 101 and PCBs with estrogenic activity. Unfortunately, the present study did not evaluate the effect of preconception exposure to PCBs in female partners, raising the possibility that including maternal data could change results for IVF outcomes. Therefore, we propose that future studies should include both maternal and paternal factors. Another limitation is that we selected only men attending the fertility clinic; therefore, our results should be applied only to that type of population.

Despite the limited number of IVF couples analyzed, our findings indicate a negative relationship between paternal DL-PCB exposure and early pregnancy outcomes. In addition, we were able to analyze intercorrelations among individual PCB congeners, which is relevant to tracking possible routes of exposure and may be relevant to designing appropriate measures in order to reduce the impact of PCB exposure on IVF outcomes. It would be interesting to carry out future studies in order to examine relationships between serum DL-PCBs and other persistent organic pollutants such as non-DL-PCBs, PBDEs and organochlorine pesticides, which can also impact IVF (Buck Louis et al., 2013; Ingle et al., 2020). We were also able to determine individual congeners of DL-PCBs as potential predictors of IVF outcomes, which deserves further attention. These findings might provide a better understanding of the effects of paternal preconception exposure to DL-PCBs on developmental toxicity.
Our findings suggest significant associations between low environmental levels of serum DL-PCBs in male partners of subfertile couples and pregnancy outcomes of IVF such as implantation, clinical pregnancy and live births. Further studies in larger population sizes with control of male and female confounders as well as technical factors related with assisted reproductive technologies are needed to confirm these results. Physicians should counsel both partners to reduce environmental exposure prior to conception.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.112248.

Author contributions


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