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Ramy Abou Ghayda
Harvard Medical School

Oleg Sergeyev
Moscow State University

Jane S Burns
Harvard T.H. Chan School of Public Health

Paige L Williams
Harvard T.H. Chan School of Public Health

Mary M Lee
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Authors

Ramy Abou Ghayda, Oleg Sergeev, Jane S Burns, Paige L Williams, Mary M Lee, Susan A Korrick, Luidmila Smigulina, Yury Dikov, Russ Hauser, and Lidia Mínguez-Alarcón



Peripubertal serum concentrations of organochlorine pesticides and semen parameters in Russian young men



Ramy Abou Ghayda^a, Oleg Sergeyev^{b,c,*}, Jane S. Burns^d, Paige L. Williams^{e,f}, Mary M. Lee^{g,h}, Susan A. Korrick^{d,i}, Luidmila Smigulina^c, Yury Dikov^{b,c}, Russ Hauser^{d,f}, Lidia Mínguez-Alarcón^{d,*}, for the Russian Children's Study

^a Division of Urology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

^b Group of Epigenetic Epidemiology, A.N. Belozersky Research Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

^c Chapaevsk Medical Association, Chapaevsk, Samara Region, Russia

^d Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA

^e Department of Biostatistics and Harvard T.H. Chan School of Public Health, Boston, MA, USA

^f Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

^g Department of Pediatrics, Sidney Kimmel Medical College, Philadelphia, PA, USA

^h Nemours AI duPont Hospital for Children, Wilmington, DE, USA

ⁱ Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

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ABSTRACT

Background: Epidemiologic literature on the relation of organochlorine pesticides (OCPs) with semen quality among adult men has been inconclusive, and no studies have prospectively explored the association between peripubertal serum OCPs and semen parameters in young men.

Objective: To evaluate prospective associations of peripubertal serum concentrations of hexachlorobenzene (HCB), β -hexachlorocyclohexane (β -HCH), and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) with semen parameters among young Russian men.

Methods: This prospective cohort study included 152 young men who enrolled in the Russian Children's Study (2003–2005) at age 8–9 years and were followed annually until young adulthood. HCB, β -HCH, and *p,p'*-DDE concentrations were measured at the CDC by mass spectrometry in serum collected at enrollment. Between 18 and 23 years, semen samples ($n = 298$) were provided for analysis of volume, concentration, and progressive motility; we also calculated total sperm count and total progressive motile count. Linear mixed models were used to examine the longitudinal associations of quartiles of serum HCB, β -HCH and *p,p'*-DDE with semen parameters, adjusting for total serum lipids, body mass index, smoking, abstinence time and baseline dietary macronutrient intake.

Results: Lipid-adjusted medians (IQR) for serum HCB, β HCH and *p,p'*-DDE, respectively, were 150 ng/g lipid (102–243), 172 ng/g lipid (120–257) and 275 ng/g lipid (190–465). In adjusted models, we observed lower ejaculated volume with higher serum concentrations of HCB and β HCH, along with reduced progressive motility with higher concentrations of β HCH and *p,p'*-DDE. Men in the highest quartile of serum HCB had a mean (95% Confidence Interval, CI) ejaculated volume of 2.25 mL (1.89, 2.60), as compared to those in the lowest quartile with a mean (95% CI) of 2.97 mL (2.46, 3.49) ($p = 0.03$). Also, men in the highest quartile of serum *p,p'*-DDE had a mean (95% CI) progressive motility of 51.1% (48.6, 53.7), as compared to those in the lowest quartile with a mean (95% CI) of 55.1% (51.7, 58.5) ($p = 0.07$).

Conclusion: In this longitudinal Russian cohort study, peripubertal serum concentrations of selected OCPs were associated with lower ejaculated volume and progressive motility highlighting the importance of the peripubertal window when evaluating chemical exposures in relation to semen quality.

* Corresponding authors at: Group of Epigenetic Epidemiology, A.N. Belozersky Research Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia (O. Sergeyev) and Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA (L. Mínguez-Alarcón).

E-mail addresses: olegsergeyev1@yandex.ru (O. Sergeyev), lmiguez@hsph.harvard.edu (L. Mínguez-Alarcón).

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1. Introduction

Meta-analyses have documented downward trends in sperm concentration and total sperm count among fertile men from 1934 to 2013 (Levine et al., 2017; Swan et al., 2000). Semen quality is used for diagnosis of male infertility (Jequier, 2011). Compared to men with good semen quality, those with poor semen quality had a higher risk of common chronic diseases (Eisenberg et al., 2014; Latif et al., 2017) and mortality (Eisenberg et al., 2014; Jensen et al., 2009), highlighting the role of semen quality as a marker for broad health beyond fertility and reproduction. Therefore, identifying modifiable factors, such as environmental and dietary exposures, that can predict semen parameters has become a major clinical and public health matter (Minguez-Alarcon et al., 2018).

Organochlorine pesticides (OCPs), such as hexachlorobenzene (HCB), β -hexachlorocyclohexane (β HCH), and 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), are persistent chlorinated compounds that have been associated with semen quality in epidemiological studies (Ayotte et al., 2001; De Jager et al., 2006; Faure et al., 2014; Paoli et al., 2015; Toft et al., 2006). Historically, they were widely used as insecticides and fungicides, and played an important role in the control of public health epidemics such as typhus and malaria (Genuis et al., 2016). Because of their ecosystem harm, environmental persistence and adverse health effects (Barber et al., 2005; Costa, 2015; UN, 2009), the use of OCPs is currently banned in most countries worldwide, especially the United States and Europe (UN, 2001; USEPA, 2016). However, they are still used as pesticides in limited regions in Asia, Africa and parts of Central and South America (FAO, 2005; Gupta, 2004; Haylamicheal and Dalvie, 2009). OCPs have low polarity and are poorly soluble in water, but are lipophilic (Longnecker, 2005; Wolff et al., 2000). Main factors that facilitate these OCPs to persist and bioaccumulate include their very long half-life, up to years, and their resistance to degradation in contaminated soils and sediments (Wauchope et al., 1992). While ingestion of contaminated food represents the main human exposure source, OCPs exposure through contaminated water, soil, dust, and, to a lesser extent air, is also possible (ATSDR, 2019; Barber et al., 2005). OCPs have demonstrated endocrine disrupting activity in experimental models (Casals-Casas and Desvergne, 2011; Gray et al., 2001). However, epidemiological studies, most of them cross-sectional, exploring the association between serum concentrations of OCPs and semen

quality in adult men have shown mixed results (Pant et al., 2013; Perry, 2008). Interestingly, no study to date has prospectively investigated the association between peripubertal serum concentrations of OCPs and semen parameters in young men. This research gap is particularly important since exposure to environmental pollutants during specific windows of testicular development (e.g. peripubertal period) has a negative impact on spermatogenesis, (Sutton et al., 2010) with potential consequences to semen quality.

Thus, we prospectively explored whether peripubertal serum concentration of HCB, β HCH and dichlorodiphenyldichloroethylene (p,p'-DDE), a metabolite of DDT, at 8–9 years are associated with semen parameters measured during young adulthood in young Russian men. Previous findings from the same cohort showed later pubertal onset among boys with higher serum HCB concentrations (Lam et al., 2014) and later age at attainment of sexual maturity among boys with higher serum HCB and β HCH (Lam et al., 2015). We have also previously reported that higher peripubertal serum dioxin concentrations were prospectively associated with lower semen parameters in this cohort (Minguez-Alarcon et al., 2017) and altered sperm DNA methylation (Pilsner et al., 2018).

2. Methods

2.1. Study population

Our study population consists of a subset of the 516 boys who were enrolled at 8 and 9 years old in the Russian Children's Study (RCS). At enrollment, each male underwent a complete physical exam and their adult guardian completed health, dietary, and lifestyle surveys. Additionally, each consented participant had blood drawn for OCPs measurements. This initial assessment was followed by yearly physical exams and questionnaires as previously described (Hauser et al., 2008; Williams et al., 2010).

Out of the 516 boys initially enrolled between 2003 and 2005, 139 (26%) were lost to follow up by the time of the semen analysis either due to death ($n = 6$) or no longer residing in the study area ($n = 129$), and 4 were not invited to participate due to severe cognitive impairment (Fig. 1). Of the 377 remaining boys, 152 declined to participate in the semen study. Thus, 225 (48%) young men provided semen samples between 2012 and 2018. Additionally, 1 participant who were

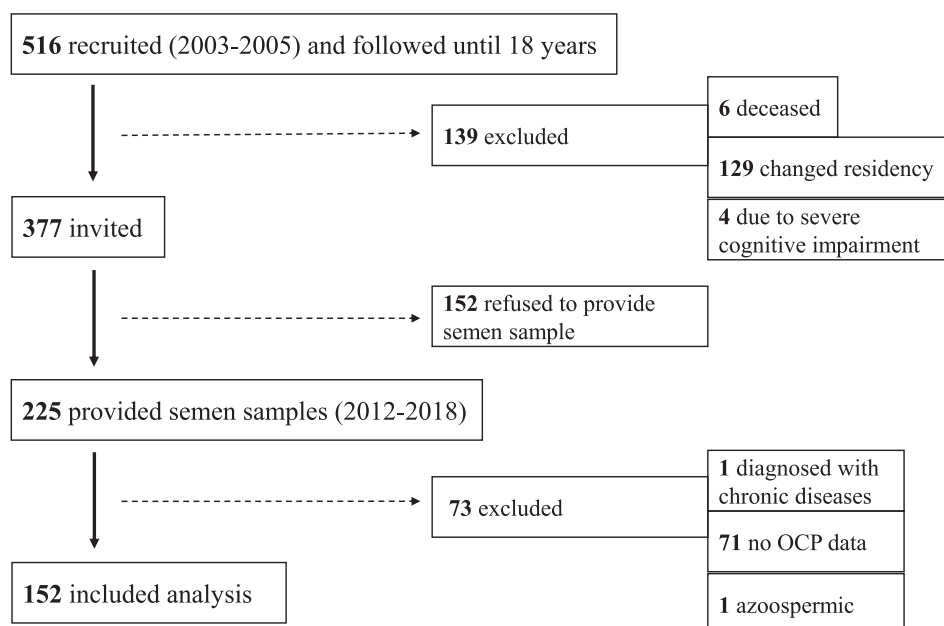


Fig. 1. Flowchart of the organochlorine pesticide and semen quality analysis in the Russian Children's Study.

diagnosed with severe chronic disease, 1 azoospermic young man and 71 subjects with missing serum OCP concentrations were excluded from the subset in the OCP analysis, leaving a final study sample size of 152 young men who provided 1 to 2 semen samples (N total = 298) at age 18–23 years (Fig. 1).

The study was approved by the Human Studies Institutional Review Boards of the Chapaevsk Medical Association (Chapaevsk, Russia), Harvard T.H. Chan School of Public Health, Brigham and Women's Hospital (Boston, MA, USA), and Nemours Health Care System (Wilmington, DE, USA). During the baseline assessment, the adult guardian/parent signed an informed consent, and each participant signed an assent before participation. Once participants were 18 years of age or older, they signed a consent form, including a separate consent for providing semen samples.

2.2. OCPs exposure assessment

During enrollment, fasting blood samples were collected, stored at -35°C , and shipped to the National Center for Environmental Health at the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) for analysis. Samples, including method blanks, and quality control samples were spiked with a mixture of $^{13}\text{C}_{12}$ -labeled pesticides as internal standards. Serum analytes were isolated by C_{18} solid-phase extraction (SPE), followed by a multicolumn automated cleanup, extraction and enrichment procedure (Sjödín et al., 2004; Turner et al., 1997). Analytes were separated using a DB-5 MS capillary column (Phenomenex, Torrance, CA, USA) and quantified using selected-ion-monitoring (SIM) high-resolution (10,000 resolving power) mass spectrometry (HRGC-ID/HRMS; Thermo Electron North America, LLC, West Palm Beach, FL, USA) (Barr et al., 2003; Patterson et al., 1987). Quantification was done by isotope dilution mass spectroscopy using calibration standards containing $^{13}\text{C}_{12}$ -labeled and unlabeled analytes. The total serum lipid content of the sample was derived from enzymatic measurements of total cholesterol and triglycerides, then calculated using the Phillips equation (Phillips et al., 1989). Quality control sample coefficients of variation combining between/within-run reproducibility were generally between 10% and 15%. All study serum concentrations of HCB, βHCH , and p,p' -DDE were above the limit of detection. All OCP concentrations were expressed on a wet-weight basis (pg/g serum) or on a lipid-normalized basis (ng/g lipid) (division of wet-weight levels by lipid concentrations).

2.3. Semen parameters assessment

Each young man included in the study was asked to contribute a semen sample upon reaching 18 years of age or older using sexual abstinence of 2–4 days. After consent as described above, they each provided 1 to 2 semen samples by masturbation inside a dedicated room next to the study Andrology Lab for a total of 298 samples. Physician recorded information regarding any viral/bacterial illness or fever in the months prior to the semen collection and date/time of last recalled ejaculation for calculation of abstinence time. The samples were stored inside an incubator at 37°C . After a maximum of one hour after sample collection, evaluation of semen parameters was carried out. Most samples (99%), however, were analyzed within a half hour of the collection. All samples were assessed by a single andrology technician (LS) and analyzed according to criteria updated by the Nordic Association for Andrology (NAFA) and European Society of Human Reproduction and Embryology–Special Interest Group in Andrology (ESHRE-SIGA) (Björndahl et al., 2010). The technician was blinded regarding serum OCPs concentrations of the subjects providing semen samples.

Semen volume was measured using a one, five, or ten mL disposable pipette. Sperm motility was evaluated by microscopic examination of the semen sample in duplicate at 400 times magnification. Results were reported following the 1999 WHO manual for the examination and

processing of human semen. Specifically, at least 200 sperm per duplicates were classified into one of 4 categories: Class A: rapidly progressive motile; Class B: slowly progressive motile; Class C: locally motile and Class D: immotile. The total percent motile sperm of the sample was calculated by summing the individual percentages of the WHO classes A, B, and C of each sample (WHO, 2010). Sperm concentration was quantified using two aliquots and Improved Neubauer Chamber Hemacytometer (INCH) at 200 times magnification under a phase contrast microscope. Duplicates for sperm concentration and motility were assessed and compared. Differences between the duplicates did not exceed the acceptance limit in any of the samples. Specifically, within-observer mean coefficient of variation (CV) in duplicates was 6.7% for sperm concentration and 4.8% for progressive motility.

2.4. Statistical analysis

We calculated medians and interquartile ranges (IQR) for participant demographics, dietary and parental characteristics that were continuous variables, and number and percentages for categorical variables. Semen parameters were reported as medians (IQR). Serum OCP distributions were reported as mean and percentiles. Because OCPs are lipophilic and because of the potential for bias, rather than modeling lipid-normalized OCPs, we instead chose to use the wet weights for OCPs and adjust for concurrently measured serum total lipids by including this as a covariate in the model (Li et al., 2013; Schisterman et al., 2005). Serum OCP concentrations (wet-weight) were divided into quartiles, and the first (lowest) quartile was used as the reference group. Total sperm count (volume \times sperm concentration), total motile sperm count (total sperm count \times % motile sperm) and total progressive motile sperm count (total sperm count \times % progressive motile sperm) were calculated. Total sperm count, sperm concentration and total motile sperm count were log-transformed to approximate a normal distribution. Linear regression models with random subject effects to account for repeated measurements within the same man were used to examine the relation between quartiles of serum OCPs concentrations and semen parameters. We compared semen parameters (total sperm count, sperm concentration, % progressive motile sperm, total progressive motile sperm count, and semen volume) among men with higher quartiles of serum OCPs concentrations to those within the lowest quartile. Predicted marginal means for these parameters were estimated as least square means (Searle et al., 1980) (adjusted for confounders at the mean level for continuous variables and for categorical variables weighted according to their frequencies) and were back-transformed to allow presentation of results in the original scale. Tests for linear trends were conducted using quartiles of serum OCPs concentrations as ordinal levels. Inverse probability of censoring weights (IPCW) were used to account for potential selection bias, based on fitting a logistic regression model to obtain predicted probabilities of having a semen sample available among the eligible men. Covariates included in the IPCW model were baseline measures of socioeconomic status and boy's health measures [birthweight, breastfeeding weeks, body mass index (BMI), total calorie intake, beer intake, household smoking, and household income] (Supplemental Table 1).

Potential confounders included in the primary models for semen parameters were selected based on *a priori* evidence from the literature and supported empirically by associations with one or more of the semen parameters and/or serum concentrations of OCPs ($> 10\%$ in change of point estimate). In addition, we included abstinence time (days) in the fully adjusted models regardless of statistical significance since this is a well-known predictor of most semen quality parameters, and thus can improve the precision of the exposure estimates in the model (Schisterman et al., 2009). Based on these criteria, in the fully adjusted models we included total serum lipids (mg/dL), BMI (kg/m^2), abstinence time (< 2 days, 2–5 days, ≥ 5 days), smoking status (yes vs no) and total caloric (kcal/day) intake, carbohydrates (% calories), and

fat (% calories) at age 8–9 years. Smoking status was collected using the question: “Have you smoked a cigarette, even a few puffs, within the past year?” prior to semen collection. To investigate the robustness of the findings, we performed sensitivity analyses excluding men who were diagnosed with cryptorchidism ($n = 3$), varicocele ($n = 5$) or orchiditis ($n = 1$) as well as further adjusting for serum concentrations of total dioxin and dioxin-like compounds toxic equivalents (total TEQs) (Minguez-Alarcon et al., 2017). We analyzed the data using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA), and two-sided p -values ≤ 0.05 were considered statistically significant.

3. Results

Of the 298 semen samples collected from the 152 young men, 146 men (96%) provided 2 samples approximately one week apart, and 6 men (4%) provided one sample. All participants were Caucasian males, with a median (IQR) age at time of semen collection of 18.2 years (18.1–19.2) and a median (IQR) BMI of 21.2 (19.2–23.4) kg/m² (Table 1). Entry characteristics of those included in the analysis were similar to those not included, although they had slightly higher BMI (Supplemental Table 1). Median (IQR) values for semen parameters are summarized in Table 2. Median (IQR) abstinence time was 2.71 days (1.88, 3.92). Over half (52%) of the semen samples were above NAF-ESHRE reference values for sperm counts (≥ 80 million) and motility ($\geq 60\%$) (Björndahl et al., 2010).

None of the OCP concentrations were below the limit of detection in the serum samples collected at entry, when the boys were 8 to 9 years old (Table 3). Lipid-adjusted medians (IQR) for serum HCB, β HCH and p,p' -DDE, respectively, were 150 ng/g lipid (102–243), 172 ng/g lipid (120–257) and 275 ng/g lipid (190–465). Spearman correlations were moderate between serum concentrations of β HCH and p,p' -DDE ($r = 0.56$) and between serum HCB and β HCH ($r = 0.51$), but weaker between serum concentrations of HCB and p,p' -DDE ($r = 0.30$). Total

Table 1

Demographic, dietary, reproductive and parental characteristics among 152 participants in the Russian Children's Study.

	Median (IQR) or N (%)
Demographics and dietary characteristics	
Age, years	18.2 (18.1, 19.2)
BMI, kg/m ²	21.2 (19.2, 23.4)
Smoking ^a , n (%)	76 (50)
Beer intake ^b , n (%)	87 (57)
Other alcohol intake ^b , n (%)	43 (28)
Total calorie intake ^b (kcal/day)	2680 (2093, 3365)
Carbohydrates ^b (% calories)	54.0 (49.7, 58.2)
Fat ^b (% calories)	34.5 (30.6, 37.8)
Protein ^b (% calories)	11.5 (10.5, 12.4)
Reproductive characteristics	
Cryptorchidism, n (%)	3 (2)
Varicocele, n (%)	5 (3)
Orchiditis, n (%)	1 (1)
Parental and residential characteristics	
Any household smoking during pregnancy ^c , n (%)	18 (12)
Parental education ^a , n (%)	
High school or less	8 (5)
College degree	83 (55)
Graduate degree or more	59 (39)

We collected BMI data from the most recent physical examination. All cases of cryptorchidism were verified after investigation of medical history and follow-up. Smoking status was based on the response to the question: “Have you smoked a cigarette, even a few puffs, within the past year?”. The questionnaire to collect smoking information was completed up to 3 years before the semen sample was collected. Diet/drink intakes and parental/residential characteristics were collected at age 8–9 years.

^a Two participants had missing data.

^b One participant had missing data.

^c Three participants had missing data.

Table 2

Semen parameters and reproductive characteristics among 152 young men contributing 298 semen samples in the Russian Children's Study.

	Median (IQR)
Ejaculated volume (mL)	2.30 (1.60, 3.40)
Sperm concentration (mil/mL)	59.1 (34.1, 97.5)
Total sperm count (mil/ejaculate)	141 (68.2, 243)
Motility (%)	64.0 (56.0, 68.0)
Progressive sperm motility (%)	55.0 (46.0, 60.0)
Total motile count (mil/ejaculate)	89.8 (39.7, 159.8)
Total progressive motile count (mil/ejaculate)	76.9 (33.3, 137)
Abstinence time (days)	2.71 (1.88, 3.92)

TEQs concentrations were moderately correlated with serum concentrations of HCB ($r = 0.56$), β HCH ($r = 0.66$) and p,p' -DDE ($r = 0.44$). The IPCW model showed that BMI at baseline, household income, beer intake and birthweight significantly differed among boys who provided a semen sample compared to those who did not in the Russian Children's Study (data not shown).

In models adjusting for serum lipids, BMI, smoking abstinence time, and intake of total calories, carbohydrates, and fats, we observed lower ejaculated volume with higher serum concentrations of HCB and β HCH, along with reduced progressive motility with higher concentrations of p,p' -DDE (Table 4). For example, men in the highest quartile of serum HCB had lower ejaculated volume than those in the lowest quartile (mean = 2.25 mL vs 2.97 mL), corresponding to a 24% decrease in ejaculated volume ($p = 0.03$). Similarly, men in the highest quartile of serum p,p' -DDE had lower progressive motility of as compared to those in the lowest quartile (51.1% vs 55.1%, $p = 0.07$). We found similar estimates in two sensitivity analyses; one excluding men who were diagnosed with cryptorchidism, varicocele or orchiditis (Supplemental Table 2) and other further adjusting for serum total TEQs concentrations (Supplemental Table 3), although associations with semen volume and motility became somewhat stronger after excluding participants diagnosed with the male reproductive conditions noted above (Supplemental Table 2).

4. Discussion

In this longitudinal study of 152 young Russian men contributing 298 semen samples, we explored prospective associations between peripubertal serum concentration of HCB, β HCH, and p,p' -DDE (at 8–9 years) and semen parameters measured approximately 10 years later. We found lower ejaculated volume with increased serum concentrations of HCB and β HCH. We also observed reduced progressive motility with increased serum concentrations of p,p' -DDE, however this association did not reach statistical significance. No other clear associations were found with any other semen parameters including sperm concentration. Previous findings from this study cohort shown associations between higher serum HCB levels and later pubertal development (Lam et al., 2014), as well as higher prepubertal serum HCB and β HCH concentrations with later age at attainment of sexual maturity (Lam et al., 2015). Serum concentrations of OCPs reported in the Russian boys at ages 8–9 years were markedly higher than children in other populations. For example, compared to U.S. boys aged 12–19 years in the 2003–2004 National Health and Nutrition Examination Survey (NHANES) (CDC, 2019), RCS participants had approximately 4 times higher serum p,p' -DDE concentrations (mean of 461 ng/g vs. 105 ng/g). The differences were even more pronounced for serum HCB concentrations, with a mean of 217 ng/g in the RCS boys compared to a reported mean of 13.3 ng/g in 2003–2004 NHANES boys. Higher serum OCP concentrations were also found among our Russian boys compared to European boys in study cohorts in Belgium (Croes et al., 2015) and Germany (Becker et al., 2006).

Exposure to OCPs and male reproductive endpoints have been a

Table 3
Distribution of serum organochlorine pesticide concentrations at baseline assessment among 152 boys in the Russian Children's Study.

	Mean (SD)	25th percentile	50th percentile	75th percentile
Wet-weight (pg/g serum)				
HCB	1074 (1539)	500	724	1159
βHCH	217 (2 7 9)	578	820	1254
p,p'-DDE	1194 (1589)	906	1386	2295
Lipid-adjusted (ng/g lipid)				
HCB	243 (3 2 3)	102	150	243
βHCH	2203 (2947)	120	172	257
p,p'-DDE	454 (5 9 6)	190	275	465

Abbreviations: HCB, hexachlorobenzene; βHCH, β-hexachlorocyclohexane; p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE).

subject of interest, however few studies have been conducted on semen quality. Experimental studies have shown evidence of a detrimental effect of OCPs on the male reproductive system and function (Bowerman et al., 1995; Guillette and Guillette, 1996; Prasad et al., 1995; Starek-Świechowicz et al., 2017). An *in vitro* study by Pant and colleagues found that exposure to γ-HCH caused significant concentration- and duration-dependent declines in sperm motility, which was later confirmed in *in vivo* models (Pant et al., 2013). An experimental animal study in Florida panthers found that exposures to p,p'-DDE, and polychlorinated biphenyls were associated with low sperm density and abnormal sperm morphology and function (Facemire et al., 1995). Organochlorine pesticides in agricultural wastes were associated with reduced penis size in Apopka Lake male alligators (Guillette and Guillette, 1996). Reduced anogenital distance, hypospadias, and cryptorchidism were found in rats exposed during the fetal period to vinclozolin, p,p'-DDE and procymidone (Gray et al., 2001). Impaired reproductive capacity has also been reported in the bald eagle (*Haliaeetus leucocephalus*) population in North America in relation to p,p'-DDE exposure (Bowerman et al., 1995). More specifically, HCH exposure was associated with a decrease in testosterone levels and abnormal decline in semen parameters in rats (Prasad et al., 1995). Furthermore, p,p'-DDE was shown to be hormonally active with the ability to penetrate the blood-testis barriers, thus potentially modulating spermatogenesis and its micro-milieu (Bush et al., 1986; Tuohimaa and Wichmann, 1985).

Whether exposure to OCPs is associated with negative semen quality in humans is less clear. Despite a multitude of studies exploring the relationship between OCPs and semen parameters in humans, findings have been inconsistent so far. These epidemiological studies varied greatly in their methods, and most of them had a cross-sectional design (Perry, 2008). OCPs such as (p,p'-DDE) and other organochlorine compounds such as 2,2',4,4',5,5'-hexachlorobiphenyl have been associated with lower sperm motility in a cross-sectional study in Ukraine (Toft et al., 2006) and reduced couples' fecundity measured as time to pregnancy in a prospective cohort (Buck Louis et al., 2013). In some cross-sectional studies, p,p'-DDE has been associated with lower sperm count and volume (Ayotte et al., 2001) and abnormal morphology (De Jager et al., 2006), and also higher levels of p,p'-DDE were found in the semen of infertile men (Pant et al., 2013). Although, Hauser and colleagues initially observed negative associations of PCBs and p,p'-DDE with sperm motility, concentration and morphology in a cross-sectional pilot study of 29 men recruited from the Massachusetts General Hospital (MGH) Andrology Laboratory (Hauser et al., 2002), they did not confirm the negative associations in a subsequent larger study of 212 men (Hauser et al., 2003). Serum p,p'-DDE was associated with a decrease in seminal volume and concentration in a cross-sectional Mexican study (Ayotte et al., 2001). This study was limited by its small sample size (N = 24 men) and extremely high serum concentrations of p,p'-DDE (up to 77900 ng/g). Multiple studies have found no relationship between OCPs and semen parameters (Bush et al., 1986;

Table 4
Adjusted mean semen parameters by quartiles of serum organochlorine pesticide concentrations among 152 young men contributing 298 semen samples in the Russian Children's Study.

	Semen Volume (mL)	Sperm Concentration (mil/mL)	Total Sperm Count (mil/ejaculate)	Progressive Motility (%)	Total Progressive Motile Count (mil/ejaculate)
HCB (pg/g serum)					
Q1 [283–497]	2.97 (2.46, 3.49)	49.8 (36.1, 68.7)	120 (79.4, 182)	51.2 (47.9, 54.8)	59.8 (37.6, 95.1)
Q2 [503–718]	2.57 (2.24, 2.90)	55.5 (45.6, 67.6)	125 (94.1, 165)	56.3 (54.2, 58.4) ^a	69.5 (51.7, 93.5)
Q3 [729–1157]	2.52 (2.06, 2.97)	46.3 (35.0, 61.3)	94.3 (68.8, 129)	52.1 (49.5, 54.8)	48.3 (34.2, 68.2)
Q4 [1160–15481]	2.25 (1.89, 2.60) ^a	56.1 (37.0, 85.1)	108 (66.6, 174)	52.8 (49.7, 55.8)	55.2 (33.1, 92.0)
P for trend	0.01	0.83	0.55	0.99	0.58
βHCH (pg/g serum)					
Q1 [222–578]	2.85 (2.31, 3.39)	47.4 (34.7, 64.8)	107 (72.7, 157)	52.5 (48.9, 56.1)	54.6 (35.7, 83.5)
Q2 [579–812]	2.86 (2.46, 3.26)	59.3 (48.6, 72.3)	151 (116, 195)	55.9 (53.5, 58.4)	83.4 (62.7, 111)
Q3 [817–1261]	2.32 (1.98, 2.65)	55.3 (39.9, 76.6)	109 (75.5, 159)	54.3 (51.8, 56.8)	58.4 (39.3, 86.9)
Q4 [1283–13732]	2.34 (1.90, 2.76) ^a	46.6 (32.6, 66.6)	89.9 (57.8, 140)	50.3 (47.7, 52.9)	44.0 (27.5, 70.4)
P for trend	0.03	0.85	0.37	0.23	0.32
p,p'-DDE (pg/g serum)					
Q1 [369–909]	2.86 (2.33, 3.38)	57.4 (42.1, 78.5)	134 (90.0, 200)	55.1 (51.7, 58.5)	72.7 (47.2, 112)
Q2 [910–1388]	2.47 (2.08, 2.85)	43.5 (30.2, 62.8)	89.3 (55.6, 143)	52.1 (49.2, 55.0)	45.3 (26.8, 76.8)
Q3 [1403–2287]	2.61 (2.20, 3.03)	53.6 (41.7, 68.8)	115 (84.4, 158)	54.6 (51.8, 57.4)	62.0 (44.5, 86.3)
Q4 [2304–27436]	2.40 (2.01, 2.79)	53.2 (38.2, 74.1)	111 (74.1, 166)	51.1 (48.6, 53.7) ^b	55.3 (35.7, 85.7)
P for trend	0.27	0.99	0.79	0.16	0.66

Data are presented as marginal means (95% CI) and models were adjusted for total serum lipids (mg/dL), BMI (kg/m²), smoking (yes, no) abstinence time (< 2days, 2–5 days, ≥5days), intake of total calories (kcal/day), carbohydrates (% calories), and fat (% calories).

^a p-value < 0.05 when compared to the lowest quartile of exposure as the referent group.

^b p-value < 0.10 when compared to the lowest quartile of exposure as the referent group. Abbreviations: HCB, hexachlorobenzene; βHCH, β-hexachlorocyclohexane; p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE).

Charlier and Foidart, 2005; Magnusdottir et al., 2005; Paoli et al., 2015; Specht et al., 2015). Magnusdottir et al. found no association between serum p,p'-DDE concentrations and male fertility defined by a poor semen quality in a retrospective case-control study (Magnusdottir et al., 2005). Also, serum concentrations of HCB did not affect semen parameters in 589 European men enrolled in a cross-sectional study (Specht et al., 2015). Another cross-sectional study by Bush et al. found no association between seminal fluid p,p'-DDE and semen quality (Bush et al., 1986).

We found that serum concentrations of HCB and β HCH were significantly associated with lower ejaculated volume, which might pose some concerns regarding fertility potential since semen hypovolemia has been identified as a risk factor for male infertility (Roberts and Jarvi, 2009). Interestingly, previous findings among men from the RCS cohort showed that higher peripubertal serum 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) concentrations and polychlorinated dibenzo-p-dioxins (PCDDs) corresponding toxic equivalents (TEQs) were strongly associated with lower sperm concentration and count, but not with ejaculated volume or progressive motility (Minguez-Alarcon et al., 2017). A possible explanation for the null results in this study on serum OCPs and sperm concentration and count, may be the competing actions of (such as dioxins) and OCPs on AhR signaling, to activate either directly or indirectly the aryl hydrocarbon receptor (AhR) signaling pathway. Dioxin-like TCDD and PCDDs exert their biological and toxicological effects by direct activation of the AhR, and other POPs, including p,p'-DDE, HCB, have shown to activate AhR signaling indirectly through inhibition of the metabolic turnover of certain endogenous AhR ligands (de Tomaso Portaz et al., 2015; Wójtowicz et al., 2011). Another explanation may be that peripubertal age is a susceptible window for OCPs impact on certain semen parameters as volume and motility, whereas it may not be for others such as sperm concentrations. Further prospective studies will be useful to confirm these hypotheses since results from this study are difficult to compare to previous cross-sectional ones in which reverse causation is a concern and thus, the potential OCPs effect on semen parameters may be underestimated. For example, not only men attending fertility centers who could have concerns about their fertility status, but also those in the general population would try to avoid pesticide exposure if it has been previously associated with lower semen quality, which in turn may decrease their serum OCPs concentrations.

This study is not without limitations. We did not account for the parents' exposure to OCPs which may have transgenerational epigenetic effects on spermatogenesis and semen parameters (Soubray et al., 2014; Stuppia et al., 2015). Also, the unusually high serum concentrations of OCPs in the RCS make generalization to other populations challenging. The main strength of this study is its prospective design, limiting the possibility of reverse causation. Other strengths include the analysis of the semen samples by a single technician who was blinded to the serum concentrations of OCPs, reducing the possibility of observer biases and variation, the collection of two semen samples for most of the young men and use of sophisticated statistical methods such as inverse probability weights to account for censoring limiting selection bias concern.

5. Conclusion

In this longitudinal Russian cohort, peripubertal serum concentrations of selected OCPs measured at 8–9 years were associated with lower ejaculated volume and progressive motility, but not sperm concentration and total count, measured later at sexual maturity. Further prospective studies are warranted to confirm these longitudinal associations.

Declaration of Competing Interest

None of the authors has any conflicts of interest to declare.

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Author Contributions

Drs Abou Ghayda, Sergeyev and Mínguez-Alarcón had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Abou Ghayda, Sergeyev, Hauser and Mínguez-Alarcón. Acquisition of data: Sergeyev, Smigulina, Dikov. Analysis of data: Abou Ghayda and Mínguez-Alarcón. Interpretation of data: All authors. Drafting of the manuscript: Abou Ghayda and Mínguez-Alarcón. Critical revision of the manuscript for important intellectual content: All authors.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2020.106085>.

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