European Journal of Obstetrics & Gynecology and Reproductive Biology 220 (2018) 39–43

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

The effect of follicullar fluid pesticides and polychlorinated biphenyls concentrations on intracytoplasmic sperm injection (ICSI) embryological and clinical outcome

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ARTICLE INFO

Article history:
Received 6 January 2017
Received in revised form 19 October 2017
Accepted 1 November 2017
Available online xxx

Keywords:
Female infertility
Toxicology
IVF
Endocrine disruptors

ABSTRACT

Objectives: The present study evaluated the correlation between the concentrations of polychlorinated biphenyls (PCBs) and pesticides in the follicular fluid (FF) obtained during intracytoplasmic sperm injection (ICSI) with the ovarian response, endometrial thickness, and embryological and clinical outcomes.
Design: Cross-sectional observational study.
Materials and methods: Women aged 20 to 38 years (300 infertile couples) presenting to a university-affiliated fertility center were approached to participate in the study. Only 150 couples that underwent ICSI for male factor infertility agreed to participate, and 94 of them had retrieved enough FF samples suitable for laboratory testing. The FF sample was obtained, centrifuged, and stored in liquid nitrogen. Two organochlorine pesticides (OCPs), Lindane and DDT;three organophosphates (OPs);chlorpyrifos; Diazinon; malathion; one Chloroacetanilide (Pretilachlor);two pyrethroids(Bioallethrin and β-cyfluthrin); and the concentrations of four PCBs, 28, 52, 138, 180, were estimated in the obtained FF samples by using gas chromatography/mass spectrometry. SPSS statistical analysis program (version 17) was used for analysis. Multiple regression analysis was used to correlate the PCBs and pesticides with ICSI outcomes.
Results: There were significant negative correlations between FF concentrations of the eight examined pesticides and the four PCBs on the endometrial thickness. However, Pretilachlor, chlorpyrifos, β-cyfluthrin, and Diazinon were the only toxic agents that negatively correlated with the number of the oocytes retrieved. Fertilization and early embryo cleavage rates were negatively correlated with Pretilachlor and β-cyfluthrin. Moreover, high concentrations of Lindane,DDT, Diazinon,and chlorpyrifos were significantly associated lower implantation rate. PCB 28 and 180 concentration in the FF was associated with a lower number of retrieved oocytes and fertilization rate, respectively. The number of implanted embryos was negatively correlated with PCB 52 FF concentration. However, the clinical pregnancy rate did not reach the level of significance.
Conclusion: Higher concentrations of any studied PCBs and pesticides are associated with thinner endometrial thickness. The higher the level of Pretilachlor, β-cyfluthrin, PCB 28 and 180, the lower the retrieval, fertilization, and embryo cleavage rates. High PCB and pesticide concentrations in the FF adversely affected embryological ICSI outcomes. However, more data are needed to evaluate their effect on the clinical outcome.

Introduction

Egypt is one of the Third World countries that still uses byproducts such as the pesticides and polychlorinated biphenyls (PCBs) of manufacturing processes that currently are considered potential causes of endocrine defects [1] These products are

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https://doi.org/10.1016/j.ejogrb.2017.11.003
0301-2115/

present in air, food, water, and the industrial field. Pesticides such as the two organochlorine pesticides (OCPs) Lindane and 1,1,1-trichloro-2,2,-bis(4-chlorophenyl)-ethane (DDT);three organophosphates (OPs),chlorpyrifos, Diazinon, malathion; one Chloroacetanilide (Pretilachlor); and two pyrethroids(Bioallethrin and β-cyfluthrin) have been used in Egypt for a long time. These organochlorine products are used in fungus and pest control [2]. PCBs are industrial and consumer products used for decades and have been banned in the United States and European countries since 1970. However, these congeners have a very long half-life in the blood of exposed subjects, ranging from 1 to 10 years, depending on the type of the congener [3]. The sources of PCBs are contaminated food and occupational, ambient, and indoor exposure [4–9]. In Egypt, some of the PCB congeners are still persistently used. These chemicals have an adverse effect on female reproductive functions and have been called endocrine disruptors [10]. They have been measured in human follicular fliud, [11–14]. Ovarian tissue [15], the uterus, amnion, placenta, and breastmilk [16,17]. In addition, reports give supporting evidence of its presence in fetuses and embryos [18]. These chemicals are also associated with high levels of recurrent pregnancy loss [19]. PCBs have adverse effects on neonatal neurological and behavioral function [20]. In utero exposure to pesticides, especially to DDT, is associated with attention and learning problems as well as a higher incidence of hypospadias in male newborns [21]. Liu and Paterson recently reported an association between prenatal exposure to pesticides and childhood obesity [22]. Still, there is conflicting data regarding the effect of these industrial toxins on assisted-reproduction outcomes.

The objective of the present study is to evaluate the correlation between the concentrations of two organochlorine pesticides (OCPs), Lindane and DDT;three organophosphate (OPs)chlorpyrifos, Diazinon, malathion; one Chloroacetanilide (Pretilachlor); and twopyrethroids(Bioallethrin and β-cyfluthrin) in addition to PCBs 28, 52, 138, 180 in the follicular fluid (FF) obtained during intracytoplasmic sperm injection (ICSI) with the ovarian response, endometrial thickness, and embryological and clinical outcomes. These byproducts are still used in Egypt, and we assumed that they may have adverse effects on assisted reproductive technology (ART) outcomes.

Materials and methods

Women aged 20 to 38 years (300 infertile couples) presenting to a university-affiliated fertility center were approached to participate in the study. The study was performed between April 2010 and July 2013. Those patients were seeking intracytoplasmic sperm injection (ICSI) for male factor. We excluded male partners who had azoospermia or severe oligospermia (less than one million). Females with endometriosis, tuberculosis, uterine endometrial lesions, or poor ovarian reserve (described as antral follicle count less than 5 and anti-mullerian hormone [AMH] less than 0.5) were excluded from the study. All patients were from Assiut, Egypt, and its rural areas. Patients consented to be included in vitro fertilization-embryo transfer (IVF-ET) and in the study. They signed an informed consent form. The study was approved by the Assiut University Faculty of Medicine Ethical Committee. Only 150 couples that underwent ICSI for male factor infertility agreed to participate, and 94 of them had retrieved enough FF samples suitable for laboratory testing. Forty-three patients became pregnant, indicating a pregnancy rate of 45.3%. Pregnancy was confirmed by quantitative human chorionic gonadotropin (HCG) levels 14 days after embryo transfer. For all patients participating in the study, a basal hormonal profile including AMH, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and E2 were performed. Combined laparoscopy and hysteroscopy were performed to exclude endometriosis and endometrial uterine lesions before the ICSI cycle. All patients received controlled ovarian stimulation, using the standard long agonist protocol. Ovum pick-up was done under general anesthesia by using transvaginal ultrasound guidance, and the follicular fluid obtained was centrifuged and stored in liquid nitrogen. Eight pesticides were tested; of them, two organochlorine pesticides (OCPs), Lindane and DDT;three organophosphates (OPs),chlorpyrifos, Diazinon, and malathion; one Chloroacetanilide (Pretilachlor); and two pyrethroids (Bioallethrin and β-cyfluthrin) were examined, and the four PCB congeners in Egypt (PCB 28, 52, 138, 180) were estimated in the obtained FF samples by using gas chromatography/mass spectrometry.

Samples preparation and analysis

The follicular fluid from each woman was collected in a sterile collecting tube. Each sample underwent centrifugation at 4000 rpm for 10 min. The separated fluid was collected and stored in liquid nitrogen for analysis. Stored samples were removed from refrigeration and equilibrated to room temperature. Human follicular fluid was added to n-hexane (1:1, v/v) and then centrifuged for 5 min at 6600 rpm at 25 °C.The hexane layerwas taken in an auto-sampler vial. Only 1 μL portion was injected into the gas chromatograph/mass spectrometry for analysis.

Chemical and solution

The standard pesticides are organochlorine pesticides (OCPs), Lindane and DDT;three organophosphates (OPs),chlorpyrifos, Diazinon, and malathion; one Chloroacetanilide (Pretilachlor); and twopyrethroids(Bioallethrin and β-cyfluthrin) with purity higher than 96.0%. The standard PCBs are PCB 28, 52, 138, and 180 with purity higher than 99.0%.

Methanol and hexane were Aldrich pure grade. Ultrapure water was used from Mille-Q system model: Milli-Q Gradient A10, Elix 3UV, and tank 60L, serial numbers F7AN24007 K, F7BN902741, USA.

Gas chromatography/mass spectrometry instrumentation

The gas chromatograph analytical system was equipped with temperature programming capability, splitless injector, capillary column, and mass quadruple spectrometry (GC/MS)detector (7890A/5975B). The computer data system was MSD ChemStation E.0201.1177, used for measuring peak area and heights from Agilent Technologies. The analytical columns used were DB-1701P (30m × 0.25 mm × 0.25 μm). Agilent part no.122-7732 was used as a primary column. Agilent part no.122-5532 was used as a secondary one. The oven temperature had been adjusted to 60 °C for 0.5 min, increased to 140 °C at 120 °C/min, 228 °C at 11 °C/min, and then to 234.22 °C for 1 min at 6.2 °C/min, 234.47 °C for 1 min at 0.25 °C/min, and increased to 260 °C for 5 min at 11 °C/min. The volume of the injected sample was 1 μL in splitless mode. The injector temperature was set at 250 °C. Helium (99.999%, purity) was used as carrier ramped flow, 0.5 mL/min for 10.9 min and then 1 mL/min to 1 mL/min for 30 min. The mass spectrometer was operated in electron impact (70 eve of ion energy), with 4.0 min solvent delay, SIM acquisition mode, mass quadruple and mass source kept at 150 °C and 230 °C (ABO El-Mali and Wahman, 2015).

Data analysis

Analysis of data was done using Microsoft® Excel® 2003 (11.5612.5606), part of Office Professional Edition 2003, product ID: 73931-640-0000106-75603.

Table 1
Baseline characteristics of the studied IVF participants.

	Studied participants (n = 94)
Age (years)	31.5 ± 6.2
Parity	1.3 ± 1.4
Body mass index (BMI)	26.1 ± 6.8
Period of infertility	5.9 ± 3.2

Linearity and range, the intraday and interday precision, method validation, limit of detection, limit of quantitation, and method detection limit were performed according to the method described by ABO El-Maali and Wahman, (2015). Correlation coefficients (r^2) were higher than 0.9980. The results for repeatability (intraday precision) show the precision of the method varied between 1.09 and −12%, and results for reproducibility (interday precision) indicate the robustness of the extraction method with mean values of relative standard deviation (RSD) around (12–19%). The LOD (limits of detection) by the empirical approach for parent compounds ranged from 0.003 for Pretilachlor, 0.005 for DDT, 0.083 for Lindane, 0.055 for diazinon, 0.024 for malathion and chlorpyrifos to 0.098 μg/L(for Bioallethrin and β-cyfluthrin)and 0.002 for PCB28, 0.012 for PCB52, 0.005 for PCB 138, and 0.01 μg/L for PCB 180.

Results

The mean ± standard deviation (SD) of our patients' ages was 31.5 ± 6.2 years. Their parity was 1.3 ± 1.4, and their body mass index (BMI) was 26.2 ± 6.8 (Table 1).

The mean + SD of the concentrations of the tested pesticides in the study participants is shown in Table 2. Unfortunately; there is no reference on the toxic cut-off level.

Table 2
Pesticide levels in follicular fluid (μg/L) of IVF participants.

Pesticides (mean + SD)	Studied participants (n = 94)
Organochlorine	
• Lindane	418.6 + 171.4
• DDT	21.1 + 3.8
Organophosphate	
• Chlorpyrifos	146.9 + 43
• Diazinon	284.7 + 108.2
• Malathion	38.5 + 9.5
Chloro acetanilide	
• Pretilachlor	2.3 + 0.5
Pyrethroid	
• Bioallethrin	29.3 +7.2
• B-cyfluthrin	32.7 +9.8
PCB	
• 28	45.5 + 9.4
• 52	370.6 + 54.1
• 138	146.2 +21.3
• 180	101.5 + 19.2

There were significant negative correlations between FF concentrations of the eight examined pesticides on the endometrial thickness (adjusted r = 0.2, P = 0.0001) (Table 3). However, Pretilachlor, chloropyrifos, B-cyfluthrin, and Diazinon were the only toxic agents that negatively correlated with the number of the oocytes retrieved (adjusted r = 0.07, P = 0.0001) (Table 3). Fertilization and early embryo cleavage rates were negatively correlated with Pretilachlor and B-cyfluthrin (P = < 005) (Table 3). Moreover, high concentrations of Lindane,DDT, Diazinon,and chloropyrifos were significantly associated with lower implantation rate (adjusted r = 0.2, P = 0.006, 0.02, 0.0001,and 0.006, respectively) (Table 3).

There were significant adverse effects from the four PCBs on the endometrial thickness (adjusted r 0.2, P = 0.0001) (Table 3). However, only PCB 28 significantly adversely affected the number of oocytes retrieved (adjusted r 0.07, P = 0.0001(Table 3).The fertilization rate was adversely affected by PCB 180 (adjusted r 0.07, P = 0.001) (Table 3).PCB 180 also adversely affected the early cleavage of embryos (adjusted r 0.1, P = 0.002) (Table 3). PCB 52 adversely affected the number of implanted sacs, diagnosed by ultrasound (adjusted r 0.2, P = 0.02) (Table 3). However, the effect on the clinical pregnancy rate does not reach the level of significance.

Discussion

PCBs and pesticides are toxic metals and organ halogens that have been named xenobiotics. The toxic effect of these chemicals has been studied in vitro and on some animals in vivo [23].

Xenobiotics have toxic effects on human reproductive function. These chemicals act as endocrine disruptors, which enter the cell and link to Ah receptors, which may interact with the cell genome. They may have either stimulatory or inhibitory effect on the function of the genome [24]. They change the functions of cell membranes and can produce oxygen free radicals [25]. Some of the organochlorinated compounds are reported to increase calcium permeability through cell membranes.

To the best of our knowledge, the current study is the first report from the Third World countries. In Egypt, the analyzed xenobiotics are still used and not banned yet. Our data showed that there is a significant adverse effect of all the studied pesticides and PCBs on follicular fluid concentrations and on the endometrial thickness. Pretilachlor and B-cyfluthrin are the two tested pesticides that have threatening effects on the number of oocytes retrieved, the fertilization rate, and the embryo cleavage rate. However, DDT had a negative impact on the implantation rate. PCB 180 had an adverse effect on the fertilization rate and the embryo cleavage rate. PCB 28 and 52 affected the number of oocytes retrieved and implanted sacs, respectively. Our data does not show any significant adverse effects on the clinical pregnancy rate. Our study is in agreement with other studies regarding the adverse effects observed during the exposure to pesticides and PCBs on reproductive outcomes [26–28] and IVF outcomes. [29,30] These reports described the studied pesticides and PCBs in their countries as having a suspicious effect on the oocyte number and fertilization and implantation rates. However, in our study, we found a strong, significant, adverse effect on these embryological outcomes after exposure to the tested pesticides and PCBs because these xenobiotics are still in use in Egypt. The levels of pesticides and PCBs are reported to be higher among Asian and African populations than among Native American adult females [31–33]. We preferred to evaluate the levels of these chemicals in the follicular fluid because it is proven to accumulate in the body fluids with a half-life of 10 to 20 years rather than in serum [34–36].

This study has some limitations. The first is the limited sample size because of the shortage of funding because it is totally

Table 3
Multiple linear regression correlation of the tested PCBs and pesticide concentrations on the ICSI outcome.

	Endometrial thickness		Number of eggs retrieved		Number of fertilized oocytes		Number of cleaved embryos		Number of sacs	
	Beta	P.value	Beta	P.value	Beta	P.value	Beta	P.value	Beta	P.value
PCB28	1.66	0.000**	0.287	0.000**	–	–	–	–	–	–
PCB52	−2.55	0.000**	–	–	–	–	–	–	8.34	0.022*
PCB138	7.44	0.000**	–	–	–	–	–	–	–	–
PCB180	−1.45	0.000**	–	–	−0.13	0.001**	−0.12	0.002**	–	–
Lindane	1.39	00000**	–	–	–	–	–	–	−1.18	0.006**
Diazinon	−0.71	0.012*	0.735	0.000**	–	–	–	–	2.94	0.000**
Malathion	−0.24	0.020*	–	–	–	–	–	–	–	–
chloropyrifos	−1.16	0.000**	−1.11	0.000**	–	–	–	–	−2.95	0.006**
Bioallethrin	0.23	0.000**	–	–	–	–	–	–	–	–
Pretilachlor	0.19	0.000**	0.294	0.000**	0.216	0.000**	0.262	0.000**	–	–
DDT	−3.71	0.000**	–	–	–	–	–	–	−2.38	0.020*
B-cyfluthrin	0.43	0.000**	0.138	0.000**	0.112	0.002**	0.151	0.000**	–	–

*Statistically significant predictors ($p < 0.05$).
**Statistically significant predictors ($p < 0.01$).
More betas → more effect.
Minus sign → negative effect (more pesticide → less thickness).

supported by the authors. Still, we do not have a robust conclusion regarding the effect of these chemicals on clinical pregnancy rates, spontaneous abortion, and live birth rates. The second limitation is that we did not evaluate the effect of these chemicals on the quality of sperm of the partners of these women. Some reports published a negative effect on the sperm quality [37,38]. Our study has some strengths, however. The prospective observational manner by collecting the follicular fluid and correlating the level of pesticides and PCBs to the cycle outcomes enabled us to produce a robust conclusion concerning the toxic effect of these chemicals on reproductive life. The assessment of oocytes retrieved, fertilization, embryo cleavage, and number of implanted embryos that are available only in IVF cycles allows for more insight in explaining early pregnancy loss.

Conclusion

Our data showed that pesticides and PCBs have adverse effects on the endometrial thickness, ooctyes retrieved, early embryo cleavage, and implantation rates during ICSI cycles in Egyptian women. The negative effects on these early embryonic stages add more insight on the process of early pregnancy loss. More data are needed to evaluate the effect on pregnancy rates, congenital anomalies, and live birth rates

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