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Organochlorine pesticides in follicular fluid of women undergoing assisted reproductive technologies from central China

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ABSTRACT

Female infertility rates have increased by approximately 4% since the 1980s. There is evidence of adverse effects on female fertility in relation to exposure of chemical pollution in recent years. Follicular fluid samples were collected from 127 woman patients (aged 20–35) who underwent assisted reproductive technologies (ART) and had no records indicating occupational exposure to OCPs. Seventeen OCPs were analyzed in this study. The results showed that methoxychlor was dominant, accounted for 13.4% of total OCPs with a mean concentration of 167.9 ± 33.9 ng/g lipid weight (lw), followed by heptachlor-epoxide, hexachlorocyclohexanes, endrin and DDT. The concentrations of OCPs in the follicular fluid samples in the present study were moderate in comparison with those reported from developed or industrialized countries. All these pollutants can accumulate in different tissues of human body through diet, drinking water and respiration. No correlation between patient age and OCP concentrations was observed in this study.

1. Introduction

Organochlorine pesticides (OCPs) were used for pest control in the past several decades throughout the world, especially in developing countries (Gupta, 2004; Wong et al., 2006). Therefore, large quantities of OCPs were widely distributed in the environment (Moon et al., 2009; Bao et al., 2012). They were well known anthropogenic and lipophilic pollutants due to their high bioaccumulation potential in fatty tissues of living organisms and amplification through the food webs. OCPs can enter animal tissues through different pathways of ingestion, dermal contact of dust and inhalation and concentrations of bioaccumulation of OCPs in animal tissues of the high level of the food chain were higher than those species of low level (El-Shahawi et al., 2010). Human exposure to OCPs is through many routes: working in or living beside OCPs factories, breathing OCPs contaminated air, drinking and taking a bath with OCPs polluted source water, eating vegetables and grains containing OCPs residues, and eating especially fish and animal meats (Smith and Gangolli, 2002; Zhao et al., 2007; Liu et al., 2010; Wang et al., 2011, 2013). A study indicated that concentrations of OCPs in sera of urban Chinese females were significantly correlated with the dietary intake of animal fat (Lee et al., 2007). OCPs accumulated in human body could cause various negative effects such as endocrine-disruption, immunological function-damage, female spontaneous abortions and preterm, and children neurodevelopment-delays (Longnecker et al., 2001; Cioroiu et al., 2010).

Endocrine-disrupting effects of OCPs had been reported (Mariscal-Arcas et al., 2010). Dich et al. (1997) revealed that long period of exposure to OCPs could evoke chronic toxicity, even though the exposure concentration is relatively low. The previous studies had reported that several OCPs could interfere with the estrogen-controlled pathway, cause weak estrogenic or anti-estrogenic response and some of them even be harmful to human nervous systems (Langer et al., 2003; Dalvie et al., 2004). Some toxic effects of OCPs on reproduction, development and immunological function of animals were observed (Cooper et al., 2004). Several studies showed that cancer risks could be induced by OCPs exposures (Moysich et al., 1998; McGlynn et al., 2006). At the same

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time, OCPs could be transferred from maternal to fetal tissues through placenta and from mother to infant through breast milk. Exposure to OCPs could also lead to some adverse effects on human productivity, including spontaneous abortions and preterm (Saxena et al., 1981), reproductive disorders of man (Martin et al., 2002; Dalvie et al., 2004) and delayed neurodevelopment during childhood (Eskenazi et al., 2006) and other negative effects.

In the past decades, attention was focused on determination and pollution levels of OCPs in human blood serum, maternal and cord serum, human milk, adipose tissue and hair and other available tissues to study human exposure and assess health risk (Pauwels et al., 2000; Chao et al., 2006; Covaci et al., 2008; Qu et al., 2010; Song et al., 2013). Spatial and temporal changes of OCPs in different human tissues were studied (Polder et al., 2008; Behrooz et al., 2009). Investigations of effects of pesticide exposure to women are relatively fewer. Negative reproductive effects such as spontaneous abortions, congenital defects and pre-maturity were reported (Arbuckle et al., 2001; Petrelli et al., 2003; Kumar, 2004). Infertility and delay in conception are another reproductive health effect from pesticide exposure (Greenlee et al., 2003), especially when exposures occurred in the early stages of gestation (Arbuckle et al., 1999).

There are many biological and other causes of female infertility such as endogenous DNA damage, diabetes, thyroid disorders, adrenal disease and exogenous factors of environmental pollution (Makar and Toth, 2002). Female infertility rates have increased by about 4% since the 1980s, mostly from problems with fecundity due to an increase in age (Maheshwari et al., 2008). Statistics showed that about 30% of the issues involved with female infertility were due to man, 30% due to woman, 10% due to both, 25% due to unexplained factors and other 5% (UK Department of Health (2009)). Since the first infant conceived with Assisted Reproductive Technology (ART) was born in the U.K. in 1978, both the use of ART to overcome infertility and the number of fertility clinics providing ART services had increased steadily in the world. The number of ART births as a percentage of total infants born in USA were about 1.5% of U.S. births, 3.1% of Australia, 4% of the Republic of Iceland, 3% of the Kingdom of Demark, 2.8% of Finland, 2.4% of Sweden, and 1.7% of Norway in 1998 (Surveillance Summaries, 2013).

The aims of this study were to 1) investigate concentrations of 17 OCPs in 127 infertile female follicular fluids, 2) assess exposure pathways, and 3) correlate the concentrations of OCPs in follicular fluids and potential effects to female infertility.

2. Materials and methods

2.1. Chemical analysis

The standard solution of OCPs at 1000 mg L^{-1} was purchased from AccuStandard, USA. A total of 17 OCPs that were analyzed included alpha-hexachlorocyclohexane (α-HCH), beta-hexachloro cyclohexane (β-HCH), gamma-hexachlorocyclohexane (γ-HCH), delta-hexachlorocyclohexane (δ-HCH), heptachlor, aldrin, dieldrin, endrin, heptachlor-exo-epoxide, endrin-aldehyde, endosulfan-sulfate, methoxychlor, p, p′-dichlorodiphenyldichloroethylene (p, p′-DDE), p,p′-dichlorodiphenyldichloroethane (p, p′-DDD), p,p′-dichlorodiphenyltrichloroethane (p, p′-DDT), α-endosulfan and β-endosulfan. All standards were prepared freshly in n-hexane. All solvents, including formic acid, water, n-hexane and dichloromethane (DCM), were chromatographic grade. Other chemicals were analytical reagent grade. All glassware were washed with K_2CrO_3/H_2SO_4 solution, baked at 450 °C for 4 h and then rinsed with n-hexane.

2.2. Sample collection and preparation

All follicular fluid samples (1 mL) were obtained in May–August 2013 from female individuals, primary or secondary infertility, visiting the Reproductive Medicine Center of Tongji Hospital, Wuhan, China. Samples were from infertile female patients who were conceiving with the ART assistance. A total of 127 woman patients had been sampled in this study. The average age were 28.65 ± 3.82 years, between 20 and 35. The number of samples and age groups were 29 in 20–25 years old, 53 in 26–30 years old and 45 in 31–35 years old. One milliliter (1 mL) of follicular fluid samples from each woman patient was obtained from Tongji hospital. Samples in glass containers were frozen at −20 °C in the dark prior to contaminant analyses. Infertility was defined as when the husband had a healthy Productivity system, but the wife could not concept after 1 year under the unprotected sexual intercourse condition or failure to give birth to a live-born child (Wulff et al., 1997).

2.3. Extraction and cleanup

Samples were extracted and cleaned according to the procedure reported (Qu et al., 2007). Five microliter (5 μL, 10 mg/L) of each surrogate standard, 2,4,5,6-tetrachloro-m-xylene (TCmX) and decachlorobiphenyl (PCB-209), was added to 1 mL of follicular fluid samples, followed by addition of 1 mL pure formic acid, 6.0 mL of n-hexane and 3.0 mL of isopropanol. The solution was transferred to a centrifuge tube and blended homogeneously. After ultrasonication for 20 min at 20 °C, it was then centrifuged at 2500 × *g* for 10 min at 20 °C. After centrifugation, the upper organic phase was transferred with a pipette and extracted with above mixed solvents for three times. The extracts were combined, dried with anhydrous sodium sulfate and weighed. After weighed lipids were re-dissolved in n-hexane, 1 mL of 98% sulfuric acid (H_2SO_4) was added to the solution and then vortexed. The solution was centrifuged at 2500 × *g* for 10 min at 20 °C to allow separation of the aqueous and organic layers. The organic phase was then transferred with a pipette and each lipid sample was extracted for three times. The extracts were combined and dried with anhydrous sodium sulfate. The organic solvent was completely dried under a gentle flow of nitrogen gas, followed by the addition of 50 μL of n-hexane. Prior to GC–MS analysis, pentachloronitrobenzene (PCNB) was added as the internal standard at a known concentration and used for the calibration of the analyzed OCPs.

2.4. Instrumental analysis

Qualitative and quantitative analysis of OCPs was carried out with an Agilent 7890 A gas chromatograph equipped with an electron capture detector (GC-ECD) and a Model 5975 mass spectrometer (MS) using electron-ionization ion source (EI) in the selected ion monitoring mode (SIM). One microliter of sample extracts was automatically injected into an HP-5MS capillary column (Agilent Technology, 30 m × 0.32 mm i.d. × 0.25 μm). Helium gas was used as the carrier gas at a constant flow of 1.0 mL/min. The injector and detector were operated at 250 °C and 300 °C, respectively. The ion source temperatures were set to 300 °C. The GC column used the temperature program started at 80 °C and held 2 min, to 190 °C via a ramp of 15 °C/min and held for 2 min, to 220 °C at 10 °C/min and kept for 5 min, and then to 260 °C at 10 °C/min and maintained for 7 min. The data were acquired and processed with Chemstation software (Hewlett–Packard).