Environ Sci Pollut Res
DOI 10.1007/s11356-014-3109-z

Dioxins in the semen of men with infertility

E. F. Galimova · Z. K. Amirova · Sh. N. Galimov

Received: 21 March 2014 / Accepted: 26 May 2014

Abstract The purpose of the present study was to assess ejaculate contamination by polychlorinated dibenzo-p-dioxins/furans in male infertility. The database of 168 infertile and 49 fertile men was included in the study. Dioxin content was determined using gas chromatography/high-resolution mass spectrometry (GC/HRMS). In the ejaculate of infertile men, the content of dioxins and furans was 2.2–2.3 times higher than in fertile donors. The maximum level of the most toxic dioxin congener was detected in pathospermia. Contamination of semen of infertile men by polychlorinated dibenzo-p-dioxins/furans supports the hypothesis about the relationship between environmental factors and reproductive health.

Keywords POP · Dioxins · Furans · Male infertility · Ejaculate

Introduction

At present, the degradation of male reproductive health is being observed in industrialized countries, and environmental pollution is supposed as the main reason for it (Sharpe 2010). Markers of anthropogenic influence are pathology of spermatogenesis, delay of puberty period, bias of sex ratio of newborns, growth of incidence of cancer of testis and prostate gland, and an increase of frequency of cryptorchidism and hypospadias (Ryan et al. 2002; Hauser et al. 2005; Delbès et al. 2010; Zanden et al. 2012). Dioxins and dioxin-like compounds (polychlorinated dibenzo-para-dioxins and furans—PCDD/Fs) refer to reprotoxic pollutants (Mocarelli et al. 2011; Schecter 2012); these substances exert their cellular and metabolic effects via the interaction with arylhydrocarbon receptor (AhR) associated with xenobiotic- (XRE) and antioxidant responsive (ARE) DNA elements (Nguyen et al. 2009). However, some researchers have doubts about both the "crisis of spermatozoid" and its relations to environmental health risks (Fisch 2008; Te Velde and Bonde 2013). The purpose of the present study was to compare the concentration of dioxin-like compounds in ejaculate with fertility pathology.

The city of Ufa was selected due to a dioxin pollution "hot point" situated close to the residential area—this is the territory of the Khimprom plant (Maystrenko et al. 1998). The plant produced 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) during the 60s until the late 80s, chlorine, and some herbicides—up to its shutdown in 2004. For 55 years of plant operation, several chlororganic products have been manufactured at large scale—2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorphenol and trichlorphenolate of copper, orthochlorphenol. In 1964–1967, the production of butyl ether of 2,4,5-trichlorphenoxyacetic acid was organized. 2,4,5-Trichlorphenol and 2,4,5-trichlorphenolate of copper were produced in 1962–1987 (Amirova et al. 2006). The polluted territory still remains a source of PCDD/Fs emission spreading over residential areas. The area of the city with the population of 1.1 million is 753.7 km^2. The large land size of the city and the prevailing winds allow for a high gradient of soil pollution—from background levels (2–4 ppt) to scores of ppb (Amirova et al. 2012). In the higher-risk zone (3 km away

Responsible editor: Leif Kronberg

E. F. Galimova (✉)
Central Research Laboratory, Bashkir State Medical University, 450000 Ufa, Russia
e-mail: efgalimova@mail.ru

Z. K. Amirova
State-financed Institution of the Republic of Bashkortostan Environmental Research and Protection Center, 450075 Ufa, Russia
e-mail: ecocnt@ufanet.ru

S. N. Galimov
Department of Biochemistry, Bashkir State Medical University, 450000 Ufa, Russia
e-mail: sngalim@mail.ru

Published online: 05 June 2014

from the plant), about 2,500 people are living in the area within 7 km—over 300,000 people. Concentration of dioxins in breast milk, blood, and adipose tissue donors from this area ranges from 28 to 62 pg/g lipid (WHO-TEQ). Increased PCDD/Fs level for inhabitants of the industrial zone confirm the high background level for the city in the whole. PCDD/Fs concentrations in blood and breast milk exceed the background level in the region by 30–40 % (Amirova and Kruglov 2001, 2005, Amirova et al. 2007).

Materials and methods

One hundred and sixty eight married men aged 22–41 years—patients of subsidiary reproductive clinics—were examined. All of them experienced infertility for 1–10 marriage years. Exclusion criteria were severe somatic pathology, diseases of the testes, and their adnexa. The control group comprised 49 fertile males having 1 to 3 healthy children and matched the infertile group by age, growth, and other parameters. Examination included spermogram analysis and identification of PCDD/Fs congeners in the ejaculate. The sperm assessment was performed according to the WHO requirements (WHO, 2010). Standard ejaculation analysis includes sperm counts, amounts of progressive motility, and abnormal forms. The study procedures were approved by the institutional review board; the written informed consent was obtained from all subjects. The data were processed with Statistica software package (StatSoft, Tulsa, OK, USA). Medians, means (M), and standard deviations (SD) were calculated; the significance of differences was assessed according to Student's t test.

Infertile men were divided into two groups. Group I comprised patients without changes in their spermogram (normospermia)—63 men. Group II consisted of 105 males with symptoms of pathospermia. Before dioxin determination, the samples of ejaculate from each group were pooled, 100 ml of the summary pool were used for analysis.

All samples had been frozen at −18 °C and kept in this state up to the time of analysis. Lipids from semen were extracted by the mixture of hexane/diethylether/ethanol. The amount of lipids was determined gravimetrically. The cleanup procedure was performed by classical methods, namely multi-layer SiO_2, Al_2O_3, and Carbopac-C/Celite columns. For the separation of high-molecular compounds in the sample preparation, the method of gel-chromatography (Envirogel TM GPC Cleanup, Waters) was used.

For measuring PCDD/Fs and DL-PCBs, HRGC/HRMS (RTX-Dioxin, 60 m, Autospec-Ultima, Waters, UK) and a series of isotope-labeled standards (CIL Corp.) were used in compliance with the US EPA 1613B methods. For calculating TEQ, the scales TEF-2005 were used.

Results and discussion

We present parameters of spermogram of infertile patients in Table 1. Spermogram parameters of 63 infertile men (group I, 38 % of all infertile men) were within WHO norms. Teratozoospermia of different degrees combined with oligo- and/or asthenospermia was diagnosed in 105 patients (group II, 62 % of all infertile men).

PCDD/Fs analysis revealed a number of specificities concerning their content and distribution in the ejaculate (Table 2).

Dioxin/furan level in seminal fluid of fertile controls was lower than that of infertile men. At the same time, there was no significant difference in total concentrations of these pollutants in infertile patients with normo- and pathospermia.

It was found that the highest concentration of 2,3,7,8-TCDD, the most toxic congener, was found in the ejaculate of men with pathospermia. Paradoxically, 2,3,7,8-TCDD made no considerable contribution to the total level of dioxin load; its share was 12 % of the equivalent dose, whereas the major part of toxicity was determined by the presence of chlorinated dibenzofurans. This aspect is not easily explained. There may be unidentified sources of PCDD/Fs emission to the environment. As a whole, the profile of PCDD/Fs congeners in the ejaculate was close to the range of PCDD/Fs in the process of incineration and does not contradict the technogenic nature of reproductive pathology.

Interpretation of our results when comparing dioxin concentration with other biological liquids is not easy. The lipid-adjusted concentration of dioxins in the sperm is higher than in breast milk and close to occupationally exposed subjects. However, the wet weight dioxin concentration in semen, taking into account low lipid levels in this liquid (1.04 g/l in pathospermia and 1.46 g/l in normospermia), appears to be considerably lower.

To understand the exposure mechanism of dioxin-like compounds on sperm, we calculated dioxin content per one gamete. We found that in patients with normospermia, there were about 16 molecules per one spermatozoon. In patients with pathospermia, however, there were 72 molecules that documents health hazard for future generation.

Table 1 Spermogram parameters of infertile males studied

Parameter	Infertile men	
	Normospermia Group I (n=63)	Pathospermia Group II (n=105)
Concentration (10^6 per mL)	47.3±2.4	12.6±2.0*
Abnormal forms (%)	41.5±5.3	90.1±5.8*
Progressive motility (%)	45.8±6.1	21.3±1.9*

*The differences between the table groups are significant according to Student's test with $p<0.05$

Table 2 PCDD/PCDF content in semen of the males studied (TEQ, pg/g lipids)

Male group	*n*	Congener							Total
		TCDD	PnCDD	HxDD	HpDD + OCDD	TCDF + PnCDF	HxCDF	HpCDF + OCDF	
Fertile donors	49	19.1	28.7	18.3	13.5	98.4	22.6	11.9	212.5
Infertile (normosprermia)	63	31.9	59.0	16.8	38.6	294.5	16.5	9.3	466.6
Infertile (pathospermia)	105	58.5	47.6	22.1	33.9	279.4	19.4	21.2	482.1

HxDD hexachlordibenzo-p-dioxin, *OCDD* octachlordibenzo-p-dioxin, *HpDD* heptachlordibenzo-p-dioxin, *OCDF* octachlordibenzofuran, *HxCDF* hexachlordibenzofuran, *PnCDD* pentachlordibenzo-p-dioxin, *HpCDF* heptachlordibenzofuran, *PnCDF* pentachlordibenzofuran, *TCDD* tetrachlordibenzo-p-dioxin

Our data show that PCDD/Fs can pass across the hematotesticular barrier. This is confirmed by the fact that their concentrations in the ejaculate are comparable with those in blood serum (Schecter et al. 1996). Taking into account the high biological activity of PCDD/Fs and the involvement of the AhR system responsible for their bioreception in providing the reproduction function (Brokken and Giwercman 2014), it is possible to suggest that dioxins as "endocrine disruptors" are directly or indirectly involved in the chain of events in reproductive organs resulting in the abnormality of the fecundating ability of spermatozoon. Our previous experiments showed the accumulation of other chlororganic pollutants—polychlorinated biphenyls—in the male reproductive system (Gromenko et al. 2008).

The toxic action of dioxins is mediated by the AhR/ARNT receptor complex. In this connection, it is important that AhR and ARNT are expressed in all seminiferous tubule stages of the human testes (Karman et al. 2012). AhR are localized in acrosome and the principal piece of the sperm flagella in normal sperm which are key regulators of reproductive processes and play an important role in normal sperm development (Hansen et al. 2014). AhR activation may result in inflammation, apoptosis, and oxidative stress in sperm leading to DNA damage (Matsumura 2009). Given that numerous metals, solvents, drugs, pesticides, and other chemical compounds and metabolites have been found in human seminal fluid (Figà-Talamanca et al. 2001), AhR may contribute to xenobiotic metabolism in sperm and may be important for fertilization. For example, 2,3,7,8-tetrachlorodibenzo-*p*--dioxin (TCDD) can increase intracellular calcium concentration (National Research Council 2013), which could impair sperm capacitation.

AhR/ARNT complex is associated with the redox-sensitive Keap1/Nrf2/ARE defense pathway (Qiang Ma and Xiaoqing He 2012). The Keap1/Nrf2/ARE redox-sensitive signal system plays the function of a molecular sensor for homeostasis shifts and is responsible for cellular protection against stress (Lushchak 2011). Disarrangement of this complicated hierarchic system in stress situations may result in its switching off, deficit of mechanisms for xenobiotics neutralization, activation of free radical processes, disturbances of homeostasis in male genital organs, and the development of reproduction pathology (Bozhedomov et al. 2009; Aitken et al. 2012).

The hypothesis that the male reproductive function deteriorated during the past 50 years is the subject of lively scientific debate. Despite the fact that there have been new studies documenting the reduction in ejaculate quality in diverse countries (Mukhopadhyay et al. 2010; Iwamoto et al. 2013; Rolland et al. 2013), on the whole, this hypothesis is not considered to be cogent enough. Meanwhile, as compared with advances in the treatment of female infertility, therapy for male infertility remains ineffective, empirical with unexpected results (Galimov et al. 2012; Ko et al. 2012) since the underlying cause of male infertility is often not clear (the so-called idiopathic infertility). Understanding mechanisms of the development of spermatogenesis pathology is necessary for the prevention of male infertility and efficiency of its treatment. The results of our research as well as data of other authors (Foster et al. 2010; Manikkam et al. 2012; Vandenberg et al. 2012) suggest effect of low concentrations of POPs on male reproductive status and call for rational preventive measures.

Conclusions

An increased level of polychlorinated dibenzo-para-dioxins and furans in the ejaculate of infertile males compared with fertile donors suggests a relationship between environmental pollution and reproduction function. The detection of a typical profile of dioxin/furan congeners corresponding to contemporary industrial manufacture confirms technogenic nature of sperm contamination.

References

Aitken R, De Iuliis G, Gibb Z, Baker M (2012) The simmet lecture: new horizons on an old landscape—oxidative stress, DNA damage and apoptosis in the male germ line. Reprod Domest Anim 47(suppl 4): 7–14

Amirova Z, Kruglov E (2001) Time history of PCDD/Fs content in blood of the population in the city of Ufa, Bashkortostan. Organohal Comp 52:269–273

Amirova Z, Kruglov E (2005) Russian dioxin "hot spot"—Ufa. Comparison with Seveso. Organohal Comp 67:2094–2098

Amirova Z, Kruglov E, Maystrenko V, Khizbullin F (2006) PCDD/Fs contamination from defoliants and chlorinated pesticides production: steps for remediation of stockpiles, soil and utilization of contaminated buildings. Organohal Comp 68:2225–2228

Amirova Z, Alexeev P, Kruglov E, Loshkina E, Khalikova N, Zhukovskaya E (2007) PCDD/Fs and PCBs-WHO levels in adipose tissue and breast milk from Russian cities. Organohal Comp 69: 1930–1933

Amirova Z, Khalilov R, Tagirova N (2012) Monitoring of high pollution zones formed due to production of 2,4,5-T, TCP and 2,4-D in the city of Ufa, Russia (1983, 1996–2012). Available at http://www.dioxin20xx.org/pdfs/2012/1307.pdf

Bozhedomov VA, Gromenko DS, Ushakova IV, Toroptseva MV, Galimov SN et al (2009) Oxidative stress of spermatozoa in pathogenesis of male infertility. Urologiia 2:51–56 (in Russian)

Brokken L, Giwercman Y (2014) Gene-environment interactions in male reproductive health: special reference to the aryl hydrocarbon receptor signaling pathway. Asian J Androl 16:89–96

Council NR (2013) Veterans and Agent Orange: update 2012. The National Academies Press, Washington

Delbès G, Hales B, Robaire B (2010) Toxicants and human sperm chromatin integrity. Mol Hum Reprod 16:14–22

Figà-Talamanca I, Traina M, Urbani E (2001) Occupational exposures to metals, solvents and pesticides: recent evidence on male reproductive effects and biological markers. Occup Med 51:174–188

Fisch H (2008) Declining worldwide sperm counts: disproving a myth. Urol Clin North Am 35:137–146

Foster WG, Maharaj-Briceno S, Cyr DG (2010) Dioxin-induced changes in epididymal sperm count and spermatogenesis. Environ Health Perspect 118:458–464

Galimov SN, Gromenko DS, Galimova EF, Gromenko YY, Iskhakov IR (2012) Effects of L-carnitine on ejaculate parameters in males from infertile couples. Urologiia 1:47–51 (in Russian)

Gromenko DS, Galimov SN, Amirova ZK, Abdullina AZ, Gromenko YY, Galimova EF (2008) Gonadotoxic effect of polychlorobiphenyls. Bull Exp Biol Med 146:70–72. doi:10.1007/s10517-008-0206-3 (in Russian)

Hansen DA, Esakky P, Drury A, Lamb L, Moley KH (2014) The aryl hydrocarbon receptor is important for proper seminiferous tubule architecture and sperm development in mice. Biol Reprod 90:8

Hauser R, Williams P, Altshul L, Korrick S, Peeples L, Patterson DG Jr, Turner WE, Lee MM, Revich B, Sergeyev O (2005) Predictors of serum dioxin levels among adolescent boys in Chapaevsk, Russia: a cross-sectional pilot study. Environ Health 4:8

Iwamoto T, Nozawa S, Mieno MN, Yamakawa K, Baba K et al (2013) Semen quality of 1559 young men from four cities in Japan: a cross-sectional population-based study. BMJ Open 3:e002222. doi:10.1136/bmjopen-2012-002222

Karman B, Hernandez-Ochoa I, Ziv-Gal A, Flaws J (2012) Involvement of the AHR in development and functioning of the female and male reproductive systems. In: Pohjanvirta R (ed) The AH receptor in biology and toxicology. Wiley, Hoboken, pp 437–466. doi:10.1002/9781118140574.ch31

Ko E, Siddiqi K, Brannigan R (2012) Empirical medical therapy for idiopathic male infertility: a survey of the American Urological Association. J Urol 187:973–978

Lushchak V (2011) Adaptive response to oxidative stress: bacteria, fungi, plants and animals. Comp Biochem Physiol C Toxicol Pharmacol 153:175–190

Ma Q, He X (2012) Molecular basis of electrophilic and oxidative defense: promises and perils of Nrf2. Pharmacol Rev 64:1055–1081

Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK (2012) Dioxin (TCDD) induces epigenetic transgenerational inheritance of adult onset disease and sperm epimutations. PLoS ONE 7:e46249

Matsumura F (2009) The significance of the nongenomic pathway in mediating inflammatory signaling of the dioxin-activated Ah receptor to cause toxic effects. Biochem Pharmacol 77:608–626

Maystrenko V, Kruglov E, Amirova Z, Khamitov R (1998) Polychlorinated dioxin and dibenzofuran levels in the environment and food from the Republic of Bashkortostan, Russia. Chemosphere 37:1699–1708

Mocarelli P, Gerthoux PM, Needham LL, Patterson DG Jr, Limonta G, Falbo R et al (2011) Perinatal exposure to low doses of dioxin can permanently impair human semen quality. Environ Health Perspect 119:713–718

Mukhopadhyay D, Varghese AC, Pal M, Banerjee SK, Bhattacharyya AK, Sharma RK, Agarwal A (2010) Semen quality and age-specific changes: a study between two decades on 3,729 male partners of couples with normal sperm count and attending an andrology laboratory for infertility-related problems in an Indian city. Fertil Steril 93:2247–2254

Nguyen T, Nioi P, Pickett C (2009) The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem 284:13291–13295

Rolland M, Le Moal J, Wagner V, Royère D, De Mouzon J (2013) Decline in semen concentration and morphology in a sample of 26 609 men close to general population between 1989 and 2005 in France. Hum Reprod 28:462–470

Ryan J, Amirova Z, Carrier G (2002) Sex ratios of children of Russian pesticide producers exposed to dioxin. Environ Health Perspect 110: A699–701

Schecter A (eds) (2012) Dioxins and Health: Including Other Persistent Organic Pollutants and Endocrine Disruptors, 3rd edn. John Wiley & Sons. 680 p

Schecter A, McGee H, Stanley J et al (1996) Dioxins and dioxin-like chemicals in blood and semen of American Vietnam Veterans from the state of Michigan. Am J Ind Med 30:647–654

Sharpe R (2010) Environmental/lifestyle effects on spermatogenesis. Philos Trans R Soc Lond B Biol Sci 365:1697–1712

Te Velde ER, Bonde JP (2013) Misconceptions about falling sperm counts and fertility in Europe. Asian J Androl 15:195–198

Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr et al (2012) Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev 33:378–455

WHO (2010) WHO laboratory manual for the examination and processing of human semen, 5th edn. WHO, Geneva

Zanden L, van Rooij I, Feitz W et al (2012) Aetiology of hypospadias: a systematic review of genes and environment. Hum Reprod Update 18:260–283