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Effects of Environmental Water Contamination in the Reproductive System of Mice*

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ABSTRACT

This study investigated the effects on the reproductive system of balb-c mice exposed to water collected from different sources near to a deactivated fertilizer waste depositary. Eighty male mice were separated in four groups: Group A (mineral water); Group B (water from the water treatment station); Group C (water from Cubatão city); Group D (water from the waste depositary region). They were exposed to water since they were weaned until they reached sexual maturity, then they were coupled with females in reproductive age and after this mating time they were sacrificed. The evaluated parameters were testicle weight, sperm analysis, pregnancy rate, sex ratio of the offspring and Sertoli cell count. The analysis of the water did not show presence of pollutants in the Group "A" and Group "C" water. Group "B" showed low level of cadmium, 3.58 ± 0.50 µg/L. Group "D" showed the presence of PAH's and high levels of lead (221 ± 16 µg/L), cadmiun (12.6 ± 1.2 µg/L) and mercury (5.3 ± 1.1 µg/L). The tests of Levene and Kolmogorov-Smirnov were employed to verify the homogeneity of the variances and the tack to the normal curve, respectively. ANOVA was used for parametric tests and Kruskall-Wallis was used for non-parametric tests, while Turkey tests were employed for multiple comparisons. There were no differences between groups in testicle weight, sperm analysis, pregnancy rate and Sertoli cell count. There was a significant reduction in sex-ratio of the offspring in Group B. This alteration cannot be explained by the cadmium levels in Group B water. In the present study we cannot associate the exposition to contaminated water from the waste depositary and reproductive alterations.

Keywords: Pollution; Reproduction; Metals; Organic Pollutants; Mice

1. Introduction

Endocrine disruptor chemicals (EDC), also known as xenostrogens, such as fertilizers, pesticides and products from plastic material degradation can lead to estrogen-like effects. Several of these products, such as biphenyl policlorades (PCB) are used since 1930's, and its chemical inertia makes it be spread out to the biosphere and can be found in several tissues from diverse species. Although some of these substances with known adverse effects have been widely banned, they persist in the environment and consequently in the food chain due to their lipophilic characteristics and their stability. They also have a high resistance to biodegradation (e.g. the half-life of DDT can be more than 50 years) [1]. Human populations throughout the world are exposed daily to low levels of environmental contaminants (pesticide residue, antibiotics, heavy metals, hormones and industrial chemicals) and the consequences of potential interactions of these compounds to human endocrine, reproductive, and immune function remain unknown [2,3]. The possibility of serious consequences such as a decline on the human population's fertility makes it an important public health issue at the start of 21st century [4].

In the last decades, the endocrine disrupters' effects in

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human being and its possible environmental impacts have been focused. Some studies have pointed out the possibility of these agents being implicated with the increase of occurrence in reproductive disturbances such as reduction in spermatogenesis [5,6] and increase of neoplasms in estrogen target organs (breast, ovaries, uterus and prostate) [7,8] both in animals and humans [9]. Other studies suggest organogenesis alteration due to exposition to these agents during the embryogenesis [10-12] and decreased indices of fertility and mating in Wistar female rats exposed to tobacco smoke during pregnancy [13].

Some other studies suggest that the quality and the quantity of human spermatozoa in semen experienced a decline, thus some have stated that the mankind is approaching to a "fertility crisis", among the factors blamed to be responsible for this possible male fertility decline: heavy metal, agricultural fertilizers and pesticides, major radiation exposure, xenoestrogen environmental contamination, and global warming [14].

Spano *et al.* [3] suggest that human dietary PCB exposure might have a negative impact on the sperm chromatin integrity of adult males. Comhaire *et al.* [15] suggest a relation between dioxin and decreased sperm motility.

In addition, some experimental studies have shown association between reproductive alterations and air pollution in experimental animals [16,17], but there is no evidence that by drinking or feeding xenostrogen contaminated water and food, adverse effects on reproductive system could be observed.

This study was developed to assess the concentration of aromatic polycyclic hydrocarbons and toxic metals in samples of water from three sites: sources near to Industrial fertilizer waste depositary, from a popular trade of bottled water and from tap water provided to Cubatão and Santos inhabitants by the mixed economy company responsible for water supply, sewage collection and treatment of 364 municipalities of São Paulo State; and toinvestigate the xenostrogen-like effect and the toxicity of this substances in the male fertility using an experimental animal model.

2. Material and Methods

A total of 80 male Balb-C mice were divided into 4 groups. They were risen up since they were weaned until they reached sexual maturity inthe same facility at the University of São Paulo Faculty of Medicine. They also received the same type of diet. Each group received water on demand from different sources: Group A (20 mice): bottled mineral water of a same brand; Group B (18 mice): water from the collecting station of SABESP (State Water Supplying Agency), in Itutinga River;

Group C (22 mice): tap water collected from the SABESP supplying system in the city of Cubatão; and Group D (20 mice): water from one of the affluent river in the Itutinga-Pilões Nucleus, State Park of Serra do Mar, which passes by the deactivated industrial waste depositary.

In the State Park of Serra do Mar Environmental Preservation located in Ituitinga-Pilões Nucleus, there was an active industrial waste depositary that worked until 1985. In that occasion, CETESB (State Environmental Protection Agency) detected a soil contamination of heavy metals and pesticides, thus, the waste depositary was deactivated since then. Nowadays, the State Park is one of water collecting locals. The collected water passes by a purifying procedure and supplies the city of Cubatão.

Water from different sources was collected weekly and stored safely regarding the temperature. Water samples of the four different sources were analyzed regarding to concentrations of toxic metals and aromatic polycyclic hydrocarbons (APH), two groups of pollutants that have been found in the Cubatão region.

As soon as they reached sexual maturity, male mice coupled with females in reproductive age for a 7 days period. Female mice received standard supplies of water and diet at the University of São Paulo Faculty of Medical. After this mating time the males were anesthetized and sacrificed by exsanguinations. The testicles were collected and weighted to observe if there was any alteration (edema, inflammation, presence of tumor, etc.), then the espermograms were done by counting the concentration of sperm from epididimus, in a Makler chamber. The sex-ratio of the offspring and the ratio of female pregnancies were also analyzed. After birth and evaluation of the offspring, the females were sacrificed.

Statistical Analysis

The descriptive analyses of all variables of the study were done. The quantitative variables were presented in terms of their values of central trend and dispersion. The percentages of pregnancy and percentage of the male sex were also presented. The tests of Levene and Kolmogorov-Smirnov were used to verify the homogeneity of the variances and then verified if it fits a Gaussian curve. For the variable that satisfied both homogeneity and normal curve parametric tests were employed (ANOVA-analyzes of variance), in other situations non-parametric tests were used (Test of Kruskall-Wallis). When differences were observed, the test of multiple comparisons of Tukey was used. To verify the association between two qualitative variables, the Qui-square test was used (SIEGEL). To compare two ratios the testto compare two proportionswas used (SIEGEL). The level of significance was of 5%. Statistical package used was SPSS 14.0 for windows.

3. Results

Table 1 presentstheconcentration of nine aromatic polycyclic hydrocarbonstested in water samples supplied for the mice groups defined in this study.

Water provided to Group D, which came from one of the affluent river in the Itutinga-Pilões Nucleus, State Park of Serra do Mar, and passes by the deactivated industrial waste depositary presented a concentration of APHs more than fivefold the concentration found in the sample of tap water provided bySABESP to Cubatão inhabitants. Also, APHs concentration on the bottled mineral water (Group A) was higher than those observed in samples from SABESP collecting station (Group B) and tap water (Group C). When we look at each APH, the concentration of Piren in sample provided to Group D was more than ten times those observed in the other water samples.

In terms of toxic metals concentrations in water samples, mercury was detected in all water samples and its concentration in the sample from the contaminated area, which was provided to Group D, was almost nine times the concentrations of mercury found in the other water samples (**Table 2**).

Table 1. Concentrations of aromatic polycyclic hydrocarbons tested in the water samples supplied for the mice group.

APHs (ng/L)	Group A	Group B	Group C	Group D
Naftalen	0.01	0.01	0.01	0.04
Acenaftilen	2.57	2.75	2.62	8.06
Fluoren	42.17	34.79	24.30	131.08
Piren	1.97	1.50	1.73	20.41
B[a]antracen	*	*	*	*
B[b]fluoranten	*	*	*	*
B[k]fluoranten	*	*	*	*
B[a]piren	*	*	0.37	0.35
DB[ah]antracen	*	*	*	*
Total APHs	46.71	39.05	29.02	159.94

*Concentration below the method detection limit.

Table 2. Toxic metals in water samples provided for the mice groups.

Heavy Metals (μg/L)	Group A	Group B	Group C	Group D
Cadmium	*	3.58 ± 0.5	*	12.6 ± 1.2
Lead	*	*	*	221 ± 16
Mercury	0.67 ± 0.13	0.62 ± 0.12	0.61 ± 0.12	5.3 ± 1.1

*Concentrations below the method detection limit.

It is important to notice that lead was only detected in the water that came from the contaminated area and cadmium concentration in the sample provided to Group D was almost fourfold the concentration found in the sample provided to Group B, which came from SABESP collecting station.

Table 3 presents the descriptive analysis of both testicles' weight according to the exposure groups.

Therewereneither intra-group nor inter-group statistical differences on testes weight.

Table 4 presents descriptive analysis of spermogram parameters (concentration and mobility) in the four experimental groups.

There were no statistical differences on concentration and mobility parameters between the four experimental groups.

Table 5 presents the distributions of offsprings according to sex in each experimental group.

Group B presented the smallest proportion of male offspring and it was statistically different from Groups A and C.

Table 6 presents the percentage of pregnancy in each one of the experimental groups.

4. Discussion

Epidemiological researches designed to explore causality of illness produced increasing evidence that exposure to toxic agents can contribute to the escalating burden of chronic disease, including congenital disorders.

Exposure to various toxicants, including EDC's, is often originated from unanticipated sources. Many common foods and fluids, for example, contain a variety of toxins including pesticide residue, antibiotics, heavy metals, hormones and industrial chemicals. The consequen-

Table 3. Descriptive analysis of testes' weight in gram according to the experimental groups.

Groups	N[1]	Mean	Standard deviation	Mínimum	Maximum
			Right testes		
A	20	0.088 g	0.010	0.073 g	0.110 g
B	18	0.090 g	0.009	0.078 g	0.110 g
C	22	0.091 g	0.010	0.074 g	0.108 g
D	20	0.091 g	0.021	0.018 g	0.117 g
			Left testes		
A	20	0.089 g	0.009	0.076 g	0.106 g
B	18	0.088 g	0.009	0.078 g	0.108 g
C	22	0.091 g	0.011	0.072 g	0.110 g
D	20	0.091 g	0.021	0.018 g	0.111 g

[1]Number of mice.

Table 4. Descriptive analysis of spermogram parameters according to the experimental group.

Groups	N[1]	Mean	Median	Standard deviation	Minimum	Maximum
			Concentration 10^6/mL			
A	18	15.06	14.45	7.01	3.30	33
B	20	14.35	16.50	6.10	0	23
C	22	16.91	15.50	5.39	9	29
D	20	14.16	13.45	7.98	0	30
			Mobile 10^6/mL			
A	18	6.55	6	4.70	1	20
B	20	8.40	10	4.22	0	11
C	22	9.50	9	4.27	4	18
D	20	6.89	6	4.24	0	13.30
Groups	N[1]	Mean	Median	Standard deviation	Minimum	Maximum
			Immobile 10^6/mL			
A	18	8.51	8.15	3.57	2.30	17
B	20	5.95	6.50	2.82	0	11
C	22	7.41	7	2.02	5	12
D	20	7.29	5.65	4.68	0	17
			Percentage of Mobility			
A	18	40.00	43.90	15.22	9.40	60.60
B	20	51.66	57.15	21.64	0	78.50
C	22	54.21	56.65	11.31	35.70	72.20
D	20	46.27	48.45	17.22	0	68.60

[1]Number of mice.

Table 5. Distributions of offsprings according to sex in each experimental group.

Groups	Male (N)	Female (N)	Total	Male%
A	21	16	37	57
B	5	23	28	19
C	34	22	56	61
D	18	26	44	41

Test to compare two proportions: Group A vs. Group B: $p < 0.01$; Group B vs. Group C: $p < 0.001$.

ces of potential interactions of these compounds to human reproductive function remain unknown.

In this study we exposed male mice to water from a deactivated chemical waste depositary (group D) and from fountainhead (group B) both located in an environmental preservation area, expecting that the water

Table 6. Percentage pregnancy average according to the groups. There were no statistical differences between the four groups analyzed in the pregnancy average parameters.

Groups	N	% Pregnancy
A	18	40
B	20	38.9
C	22	54.5
D	20	55

coming from these sources could have been contaminated by heavy metals, pesticide residue, antibiotics and hormones. Two other groups were exposed to mineral water (group A) and water from the SABESP Supplying system to the city of Cubatão (group C). SABESP is a government water supplying system that is responsible for the provision of treated water to more than 80% of the population of the city.

There was no indication of toxicity of the group D water to the reproductive-related end points examined in this study. In group B the number of males in offspring was significant lower than other groups but the analysis of the water didn't show the presence of substances in concentrations that could cause the lowering of male percentage in offspring. Although speculation could be made about the source of water, since this fountainhead is also located in the same environmental preservation area as the water of group D (industry waste depositary). The only plausible conclusion is that the water of group B was finely treated by SABESP turning into water of group C, in which we could observe a reduced percentage of APHs and heavy metals, compared with water from other resources.

Although there are studies showing a reduced number of males in the offspring in animals exposed to air pollution [17,18], there is still no studies that could convincingly establish a causal relationship between presence of EDC in the water and reduced male percentage in offspring of animals exposed to the contaminated water. The spermatozoid containing the Y chromosome is more fragile than the spermatozoid X to the effects of chemicals, drugs and physical alterations and this fact could explain the alteration in the sex-ratio reducing the number of males in the offspring. Unfortunately, we could not, in this study, reproduce the results reached by those studying air pollution. Perhaps giving to the animals water containing additional EDC's, carefully prepared in laboratory in controlled conditions, instead of water collected in natural sources could produce more convincing results, but, in order to verify if water in natural sources and collected to be treated and distributed to the inhabitants of Cubatao could contain harmful substances, we preferred to use water from these sources instead of water containing contaminants prepared in laboratory.

Other possibilities for the poor results presented in this study are the fact that the EDC's effects are cumulative and remain throughout generations, if our study could cover for more than two or three generations of mice, maybe we could observe some sex-ratio alteration from second or even more generation of mice. The water collected and given to group D was supposed to be contaminated by xenostrogens, perhaps the metal and EDC concentrations were not enough to provoked adverse effects we hoped to observe.

5. Conclusion

Although evidences suggest some alteration in sex ratios and adverse effect in reproductive system in mice due to endocrine disruptors' exposure, we did not find convincing results to support the results found in other studies. Further studies are needed to better establish the possible association between contaminated water and reproductive alterations.

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