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EFFECTS OF SODIUM FLUORIDE IN DRINKING WATER ON FERTILITY IN FEMALE MICE

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SUMMARY: Adult female mice were allowed *ad libitum* access to tap water containing 0, 100, 200 and 300 ppm sodium fluoride (NaF) for either 4 weeks or 12 weeks. The effect of NaF exposure on fertility was assessed in terms of the numbers of pregnant mice, implantations, viable fetuses, and resorptions. Exposure to NaF for 4 weeks did not have much effect on fertility, although there was a significant increase in the relative ovary weights and a decrease in the embryo weights in mice exposed to 300 ppm NaF. However, exposure to NaF for 12 weeks resulted in a significant reduction in the percentage of pregnancies at all concentrations used. Mice exposed to 200 and 300 ppm NaF showed a significant increase in relative ovary weights and a decrease in the number of viable fetuses. Furthermore, exposure to 300 ppm NaF for 12 weeks resulted in a significant decrease in number of implantations. These results indicate that long-term exposure of female mice to NaF causes adverse effects on the reproductive system and fertility.

Keywords: Female mice, Fluoride and fertility, Reproductive effects, Sodium fluoride.

INTRODUCTION

Fluoridation of water supplies is still practiced in many areas of the world even though there is increasing evidence against much dental benefit from water fluoridation.[1] The results of a national survey of U.S. school children showed no significant differences in caries incidence among fluoridated, nonfluoridated, and partially fluoridated communities.[2] More recently, it has been reported that children living in a nonfluoridated control city in New York had slightly less tooth decay overall in comparison with children in a long-time fluoridated city in the same state.[3]

In addition to the lack of evidence of benefit in decreasing the prevalence of dental caries, exposure to fluoride has been reported to cause toxic hazards, for example to people with impaired kidney function, as well as chronic toxic hazards of gene mutations, cancer, neurotoxicity, bone pathology, dental fluorosis, and reproductive effects.[4-19]

Epidemiological studies have also shown that there is an association of decreasing total fertility rate with increasing fluoride levels in drinking water.[13] Other studies have revealed decreased testosterone concentrations in skeletal fluorosis patients and in males living in areas endemic for fluorosis.[14] Additionally, *in vitro* studies have demonstrated that exposure of human sperm to NaF resulted in a significant decline in sperm motility.[12]

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Studies in different animal species have provided evidence for reproductive toxicity of sodium fluoride.[6,15-19] The emphasis so far has been on the effect of sodium fluoride on male fertility, although we have recently reported on the fetotoxic effects of ingestion of NaF for 30 days in the female rat.[18] Thus, the current study is part of ongoing research and investigates the effect of both short term (4-week) and long term (12-week) exposure to NaF on fertility in female mice.

MATERIALS AND METHODS

Animals: Eighty adult female Swiss mice (60 days old) were used in this study. They were raised in the animal house unit in the Faculty of Medicine at Jordan University of Science and Technology under a controlled temperature of 21±1.0°C on a 12-hr light/dark cycle. Food (manufactured by the Faculty of Veterinary Medicine at Jordan University of Science and Technology, Irbid, Jordan, according to standard recipes) and water were supplied *ad libitum*. Since both the diet and water had low levels of fluoride (F), our calculations of F intake are based solely on the sodium fluoride (NaF) added to the drinking water.

Administration of sodium fluoride: Mice were exposed for 4 weeks or 12 weeks to different concentrations (100, 200, and 300 ppm) of NaF (Sigma Chemical Company, St Louis, MO, USA) dissolved in tap water. For either exposure time, mice were randomly divided into four groups of 10. The first group served as the control and the animals were allowed *ad libitum* access to tap water without any added NaF. The other three groups were allowed *ad libitum* access to tap water containing either 100, 200, or 300 ppm NaF.

Fertility test: Animals were observed daily from the first day of exposure to NaF for clinical signs of toxicity. Their water consumption was measured every day and the body weights every week. The effect of NaF ingestion on the occurrence of implantation was estimated in the mice and in their control counterparts after the appropriate time of exposure. Treated mice and their control counterparts were divided randomly into groups of two animals each and housed with a sexually mature untreated male of proven fertility for ten days. During this period, at least two estrous cycles should have elapsed.[20] One week after the removal of untreated males, the treated females and their control counterparts were killed by cervical dislocation under light ether anesthesia. During autopsy the following measurements were recorded: number of pregnant females, number of implantations, number of viable fetuses, and number of resorptions. Furthermore, the maternal body, uterus, and ovary weights were measured, and the embryo weights were also recorded.

Statistical analysis: The data were analyzed with the Student '*t*' test and the Fisher exact test using Minitab statistical package (Minitab Release 9, Minitab Inc., State College, PA, USA).

RESULTS

Exposure levels and toxicity of NaF: The actual doses that the animals received based on the water consumption per kg body weight per day were 9.52 - 27.70 mg NaF for the three groups after 4 weeks of exposure (Table 1a) and 5.82 - 18.85 mg for the three groups after 12 weeks of exposure (Table 1b).

None of the animals in the 4-week exposure group showed any clinical signs of toxicity. However, for the 12-week exposure group, 1 animal out of 10, 2 animals out of 10, and 2 animals out of 10 died in the groups exposed to 100, 200, and 300 ppm NaF, respectively.

Table 1a. Average water consumption of female mice exposed to sodium fluoride for 4 weeks

Treatment	No. of animals	Water consumption (mL/day)[a]	Dose of NaF (mg/kg/day)[b]
Control	10	3.35 ± 0.05	0
NaF (100 ppm)	10	4.27 ± 0.07	9.52 ± 0.17
NaF (200 ppm)	10	4.15 ± 0.49	18.43 ± 2.2
NaF (300 ppm)	10	3.35 ± 0.37	27.70 ± 3.03

[a]Results are expressed as means ± SEM.
[b]Dose of NaF intake is based solely on added NaF in the drinking water.

Table 1b. Average water consumption of female mice exposed to sodium fluoride for 12 weeks

Treatment	No. of animals	Water consumption (mL/day)[a]	Dose of NaF (mg/kg/day)[b]
Control	10	2.76 ± 0.09	0
NaF (100 ppm)	9	2.49 ± 0.89	5.82 ± 0.80
NaF (200 ppm)	8	2.23 ± 0.12	12.74 ± 1.11
NaF (300 ppm)	8	2.26 ± 1.1	18.85 ± 0.73

[a]Results are expressed as means ± SEM.
[b]Dose of NaF intake is based solely on added NaF in the drinking water.

Effect of NaF on fertility: Table 2a shows the effect of ingestion of different concentrations of NaF for 4 weeks on the fertility of female mice. There were no significant differences between the control and the NaF treated groups in regard to the number of females becoming pregnant nor on the

number of implantations. The number of viable fetuses was slightly lower in the NaF treated groups.

Table 2b shows the effect of ingestion of different concentrations of NaF for 12 weeks on the fertility of female mice. There were significant decreases in the percentage of pregnant mice in the NaF treated groups ($p<0.05$ for 100 ppm NaF, $p<0.001$ for the 200 ppm NaF, and $p<0.05$ for the 300 ppm NaF), in comparison to their control counterparts. Furthermore, exposure to NaF resulted in a decrease in the number of implantations at all concentrations used but the decrease was statistically significant only in the group exposed to 300 ppm NaF ($p<0.05$). There was also a significant reduction in the number of viable fetuses in mice exposed to 200 and 300 ppm NaF ($p<0.05$).

Table 2a. Effect of 4-week exposure to sodium fluoride on fertility of female mice

Treatment	No. of pregnant females	No. of implantations[a]	No. of viable fetuses[a]	No. of resorptions/No. of implantations
Control	10/10	10.0 ± 0.60	10.0 ± 0.60	1/100
NaF (100 ppm)	10/10	9.5 ± 0.54	9.5 ± 0.54	1/95
NaF (200 ppm)	10/10	9.4 ± 0.92	9.4 ± 0.92	2/94
NaF 300 ppm)	10/10	9.2 ± 0.25	9.2 ± 0.25	3/92

[a]Results are expressed as means ± SEM.

Table 2b. Effect of 12-week exposure to sodium fluoride on fertility of female mice

Treatment	No. of pregnant females	No. of implantations[a]	No. of viable fetuses[a]	No. of resorptions/No. of implantations
Control	9/10 (90%)	7.33 ± 0.85	7.33 ± 0.85	0/66
NaF (100 ppm)	6/9 (66%[†])	5.50 ± 1.00	5.00 ± 1.10	3/33
NaF (200 ppm)	4/8 (50%[‡])	5.25 ± 1.10	4.00 ± 0.71[*]	5/21
NaF 300 ppm)	5/8 (62%[†])	5.00 ± 0.32[*]	4.20 ± 0.86[*]	4/20

[a]Results are expressed as means ± SEM.

[*]$p<0.05$ significantly different from the control group (Student's *t* test).

[†]$p<0.05$, significantly different from the control group (Fisher exact test). [‡]$p<0.001$.

Effect of NaF on maternal organ weights and embryo weights: Table 3a shows that ingestion of 300 ppm NaF for 4 weeks resulted in a significant

increase in the relative ovary weight ($p<0.05$). There was also a statistically significant increase in the embryo weights of animals exposed to 200 and 300 ppm NaF ($p<0.05$ and $p<0.01$, respectively), in comparison to the control counterparts. There were no significant differences in the uterine weights of animals exposed to NaF for 4 weeks in comparison to their controls.

Table 3b shows that ingestion of 200 and 300 ppm NaF for 12 weeks also resulted in a significant increase in the relative ovary weights ($p<0.05$). Animals exposed to 300 ppm NaF showed a significant increase in the embryo weights ($p<0.001$) in comparison to the control counterparts. No significant differences were observed in the uterine weights of animals exposed to NaF for 12 weeks in comparison to their controls.

Table 3a. Effect of 4-week exposure to sodium fluoride on maternal organ weights and embryo weights

Treatment	No. of females	Bwt on day of sacrifice (g)	Ovary weight (mg/10 g Bwt)[a]	Uterus weight (mg/10 g Bwt)[a]	Embryo weight (g)
Control	10	45.77 ± 1.20	10.09 ± 0.69	201.5 ± 6.0	0.746 ± 0.041
NaF (100 ppm)	10	45.03 ± 1.80	9.73 ± 0.59	204.9 ± 4.6	0.812 ± 0.090
NaF (200 ppm)	10	42.57 ± 6.03	9.92 ± 0.69	209.0 ± 11.0	1.014 ± 0.064*
NaF (300 ppm)	10	36.28 ± 1.10	12.53 ± 0.65*	183.2 ± 7.8	1.172 ± 0.053†

[a]Relative weights. Results are expressed as means ± SEM.

*$p<0.05$, †$p<0.01$ significantly different from the control group (Student's *t* test).

Table 3b. Effect of 12-week exposure to sodium fluoride on maternal organ weights and embryo weights

Treatment	No. of females	Bwt on day of sacrifice (g)	Ovary weight (mg/10 g Bwt)[a]	Uterus weight (mg/10 g Bwt.)[a]	Embryo weight (g)
Control	10	45.24 ± 1.50	11.5 ± 1.20	142.3 ± 8.70	0.804 ± 0.039
NaF (100 ppm)	9	42.78 ± 2.40	11.3 ± 1.20	123.8 ± 16.0	0.640 ± 0.100
NaF (200 ppm)	8	35.00 ± 0.97	17.4 ± 2.20*	132.0 ± 17.0	0.637 ± 0.084
NaF (300 ppm)	8	39.15 ± 2.00	14.3 ± 0.30*	151.3 ± 12.0	1.154 ± 0.047†

[a]Relative weights. Results are expressed as means ± SEM.

*$p<0.05$, †$p<0.001$ significantly different from the control group (Student's *t* test).

DISCUSSION

We have investigated the effect on fertility of 4-week and 12-week exposure of adult female mice to 100, 200, and 300 ppm NaF administered in

drinking water. The animal model used in this study has been used previously in several studies to assess the adverse effects of different compounds on reproduction in laboratory animals.[21-22] The concentrations of NaF used in the current study were chosen according to previous studies.[23]

We have shown that exposing adult female mice to NaF in drinking water for 4 weeks had no significant effect on their fertility. However, there was a statistically significant increase in the relative ovary weights at 300 ppm and in embryo weights at 200 and 300 ppm. In line with these results, exposure of mice for 12 weeks resulted in a significant increase in the relative ovary weights at both 200 ppm and 300 ppm NaF and in embryo weights at 300 ppm. Thus short-term and chronic exposure to NaF results in increased embryo weights at the highest dose level of 300 ppm. This is in contrast to a recent report on the developmental evaluation of NaF on rats and rabbits where no biologically relevant effects on fetal body weight were found.[24] The increase in ovarian weights in mice exposed to NaF needs to be clarified by histological studies. This will help to elucidate whether the weight increase in the ovaries observed in this study is due to hyperplasia or hypertrophy of a specific tissue compartment in this organ. Any increase in the weight of reproductive organs is under hormonal control. The results, therefore, suggest a disturbance of the reproductive endocrine functions, possibly with multiple sites of toxicity along the hypothalamic-pituitary-ovarian-uterine axis.

The main finding of the current study was the significant reduction in the occurrence of pregnancy in mice exposed to NaF for 12 weeks. This decrease may be due to alteration of the reproductive endocrine functions leading to decreased secretion of progesterone which is needed for endometrial alteration at the time of implantation and is necessary for successful pregnancy. This may also explain the significant decrease in the number of implantations resulting in a decrease in the number of viable fetuses. We are now investigating the effect of NaF exposure on serum progesterone levels.

In line with the current study, other reports in the literature have also suggested that NaF adversely affects fertility in animals. A study in owls has shown that chronic dietary NaF administration can cause slight to moderate reproduction disorders.[15] Administration of NaF, at 200 ppm, resulted in lower egg weights and lengths and in a 10% reduction of the weights of day-one hatchlings in comparison to their control counterparts. Another study in foxes has shown that reduction in dietary fluoride levels resulted in decreased neonatal mortality and increased kit production.[25] Furthermore, a high fluoride concentration in drinking water has also been linked with decreased human birth rates.[13]

In conclusion, the results of the current study suggest that ingestion of NaF by adult female mice causes adverse effects on fertility and reproduction.

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