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FERTILITY EFFECTS OF SODIUM FLUORIDE IN MALE MICE

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SUMMARY: Sexually mature male Swiss mice were exposed at 60 days of age to 100, 200 and 300 ppm sodium fluoride (NaF) in their drinking water for 4 weeks or 10 weeks. The effect of NaF exposure on fertility was assessed by breeding these males with untreated female mice after the exposure periods. Fertility was significantly reduced at all three concentrations by exposure for 10 weeks but not for 4 weeks. The number of implantation sites and viable fetuses was significantly reduced in females mated with males that had ingested NaF at a concentration of 200 ppm for 10 weeks. Relative weights of seminal vesicles and preputial glands were significantly increased in mice exposed to 200 and 300 ppm NaF for 4 weeks but not in mice exposed for 10 weeks. These results indicate that long-term ingestion of NaF adversely affects fertility in male mice.

Keywords: Fluoride and fertility, Fertility reduction, Male mice, Sodium fluoride.

INTRODUCTION

The effect of fluoride on male and female fertility has become an area of growing concern. Various studies show that fluoride causes adverse effects on both male and female fertility.[1-3] Fluoride intake has been linked to lowered human birth rates[1] and decreased testosterone concentrations.[3] Infertility was found in an area of India where fluorosis is highly endemic.[2,4] Furthermore, *in vitro* exposure of human sperm to fluoride produced a significant decline in sperm motility.[2]

Sodium fluoride has been tested in fertility studies in several species of laboratory animals. Some studies indicate no effects on reproductive function and development[5-9] whereas others reveal that exposure to sodium fluoride causes reproductive toxic effects.[10-13] The reported reproductive toxic effects include increases in numbers of abnormal spermatozoa,[11] loss of spermatogenesis,[12] and interference with steroidogenesis.[13]

In order to gain further insight into the effect of sodium fluoride on male fertility, the present study was undertaken to investigate the effects of sodium fluoride on fertility in male mice.

MATERIALS AND METHODS

Animals: Eighty 60-day-old adult male Swiss mice were used in the experiments. They were raised in the animal house unit in the Faculty of Medicine at Jordan University of Science and Technology at a controlled temperature of 21 ± 1° C on a 12-h 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*. The

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diet had very low levels of fluoride (F), and the tap water contained 0.3 ppm F. Therefore, our calculations of F intake are based solely on the sodium fluoride (NaF) added to the drinking water.

Administration of sodium fluoride: Sodium fluoride (Sigma Chemical Company, St Louis, MO, USA) was dissolved in tap water (0.3 ppm), at a concentration of 100, 200 and 300 ppm NaF. The exposure of mice was conducted in two experiments: in one experiment the mice were given NaF in their drinking water for a period of 4 weeks. For this experiment, 40 of the mice were randomly divided into four groups of 10. The first group served as the control and was allowed *ad libitum* access to tap water without any added NaF for 4 weeks. The other three groups were allowed *ad libitum* access to tap water containing either 100, 200 or 300 ppm NaF for the same period. In the second experiment the other 40 mice were randomly divided into four groups of 10 and treated the same as in the first experiment except that the exposure period was 10 weeks.

Fertility test and examination of fetuses: Animals were observed daily for clinical signs of toxicity from the first day of exposure to NaF. Their water consumption and body weight were measured every week. After the exposure period each male was placed in an individual cage with two virgin untreated females of the same strain and *ad libitum* access to food and untreated tap water. They were left together for ten days during which two estrus cycles should have elapsed.[14] The males were then removed, and 10 days later the females were killed by cervical dislocation under light ether anesthesia, and the following measurements were recorded: number of pregnant females, number of viable fetuses, and number of embryo resorptions.

Body and organ weights: The exposed male mice and their control counterparts were sacrificed at the end of the exposure period (4 or 10 weeks) plus the 10-day mating period. Body weight and weights of paired testes, seminal vesicles (stripped of fluid), and preputial glands were recorded.

Statistical analysis: Data are expressed as mean ± SD. Differences between control and NaF-exposed groups were analyzed using either the Fisher exact test or Student's t test. A p value less than 0.05 was considered significant.

RESULTS

Effect of NaF on water consumption: Water consumption of the exposed and the control groups was measured every week, and the average daily water consumption for each animal was calculated. The data depicted in Tables 1 and 2 demonstrate that ingestion of NaF (100, 200, or 300 ppm concentration) did not significantly affect the average daily water consumption per animal. The average doses of NaF that the 4- and 10-week exposed animals received based on water consumption per kg body weight per day are shown in Tables 1 and 2. None of the control nor any of the NaF groups showed any clinical signs of toxicity when the exposure period was 4 weeks. In the 10-week exposed groups, however, 2 animals out of 10 and 3 out of 10 died during the last week of the exposure to 100 and 300 ppm NaF, respectively.

Table 1. Effect of 4 weeks ingestion of sodium fluoride on average daily water consumption in male mice

Treatment	No. of animals	Water consumption (mL/day)	NaF from drinking water (mg/kg/day)[a]
Control	10	3.55 ± 1.06	-
NaF (100 ppm)	10	4.38 ± 0.83	12.35 ± 3.16
NaF (200 ppm)	10	3.90 ± 0.75	21.80 ± 6.45
NaF (300 ppm)	10	3.68 ± 0.78	39.19 ± 5.19

Data are expressed as means ± SD. [a]The calculations of F intake are based solely on the NaF added to the drinking water.

Table 2. Effect of 10 weeks ingestion of sodium fluoride on average daily water consumption in male mice

Treatment	No. of animals	Water consumption (mL/day)	NaF from drinking water (mg/kg/day)[a]
Control	10	3.01 ± 0.82	-
NaF (100 ppm)	10	3.23 ± 1.16	8.85 ± 3.63
NaF (200 ppm)	10	2.92 ± 1.06	15.64 ± 7.01
NaF (300 ppm)	10	2.73 ± 1.03	27.25 ± 13.92

Data are expressed as means ± SD. [a]The calculations of F intake are based solely on the NaF added to the drinking water.

Effect of NaF on organ weights: Mice exposed to 200 and 300 ppm NaF for 4 weeks showed a statistically significant increase in the relative weights of preputial glands ($p<0.01$ and $p<0.001$, respectively) (Table 3). The relative testes weights were also significantly increased in the group exposed to 200 ppm NaF for 4 weeks. On the other hand, animals exposed to NaF for 10 weeks showed no significant effects on the relative weights of any reproductive organ investigated in this study (Table 4).

Effects of NaF on fertility of male mice: Table 5 demonstrates that the exposure of adult male mice to NaF for a period of 4 weeks had no effect on male fertility (the number of pregnant females impregnated by exposed males was very close to the number impregnated by the unexposed control males). The number of resorptions, however, was significantly increased in females impregnated by males that had been exposed to NaF at 100 ppm ($p<0.005$), 200 ppm ($p<0.05$) and 300 ppm ($p<0.005$).

Table 6 demonstrates the effects of 10 weeks ingestion of NaF on male fertility. The number of females that became pregnant was significantly reduced when they were mated with males exposed to 100 ppm ($p<0.0005$), 200 ppm ($p<0.0001$) and 300 ppm ($p<0.00001$) NaF. The number of implantations was significantly decreased in females impregnated by males which had ingested

200 ppm NaF ($p<0.01$). Furthermore, the number of viable fetuses was also reduced in pregnant females impregnated by males exposed to 200 ppm NaF.

Table 3. Effect of 4 weeks ingestion of sodium fluoride on organ weights

Treatment	Weight on day of sacrifice (g)	Testes (mg/10 gm B.wt.)[a]	Seminal vesicles (mg/10 gm B.wt.)[a]	Preputial glands (mg/10 gm B.wt.)[a]
Control	33.87 ± 1.98	70.91 ± 6.46	55.80 ± 5.58	17.18 ± 4.78
NaF (100 ppm)	36.04 ± 2.27	64.57 ± 8.34	59.90 ± 6.63	19.30 ± 1.80
NaF (200 ppm)	39.26 ± 2.94	66.80 ± 10.18	66.71 ± 9.55*	23.39 ± 4.36*
NaF (300 ppm)	31.93 ± 3.41	66.73 ± 9.22	48.54 ± 8.80	27.68 ± 6.39†

[a]Relative weights. Data are expressed as means ± SD.

*p<0.01, †p<0.001 significantly different as compared to control values (Student's *t* test).

Table 4. Effect of 10 weeks ingestion of sodium fluoride on organ weights of male mice

Treatment	No. of animals	Weight on day of sacrifice (g)	Testes (mg/10 gm B. wt.)[a]	Seminal vesicles (mg/10 gm Bwt)[a]	Preputial glands (mg/10 gm Bwt)[a]
Control	10	34.78 ± 2.27	63.68 ± 7.68	72.78 ± 18.91	20.63 ± 5.72
NaF (100 ppm)	8	39.75 ± 1.21	59.89 ± 5.90	60.05 ± 5.56	21.68 ± 8.09
NaF (200 ppm)	10	36.78 ± 6.28	60.85 ± 10.42	58.92 ± 12.61	22.58 ± 1.72
NaF (300 ppm)	7	33.74 ± 7.67	59.37 ± 20.62	74.45 ± 27.93	20.34 ± 1.49

[a]Relative weights. Data are expressed as means ± SD.

Table 5. Effect of 4 weeks ingestion of sodium fluoride on fertility of male mice

Treatment	No. of males	No. of females	Pregnant females	No. of implantations[a]	No. of viable fetuses[a]	Resorptions/ implantations
Control	10	20	20/20 (100%)	8.75 ± 1.12	8.75 ± 1.12	0/175
NaF (100 ppm)	10	20	18/20 (90 %)	8.67 ± 1.50	8.22 ± 1.80	8/156†
NaF (200 ppm)	10	20	19/20 (95%)	8.11 ± 1.85	7.84 ± 2.14	6/155*
NaF (300 ppm)	10	20	17/20 (85%)	8.41 ± 2.72	8.00 ± 3.08	7/143†

[a]Results are expressed as means ± SD. *p< 0.05, †p < 0.005 significantly different as compared to control values (Fisher's exact test).

Table 6. Effect of 10 weeks ingestion of sodium fluoride on fertility of male mice

Treatment	No. of males	Dead males	Mated females	No. of pregnant females[a]	No. of implantations[a]	No. of viable fetuses[a]	Resorptions/ implantations
Control	10	0	20	20/20 (100%)	7.80 ± 1.61	7.80 ± 1.61	0/156
NaF (100 ppm)	10	2	16	8/16 (50%)*	8.22 ± 1.86	8.11 ± 1.76	1/74
NaF (200 ppm)	10	0	20	9/20 (45%)†	5.78 ± 1.80§	5.33 ± 1.80‖	4/52
NaF (300 ppm)	10	3	14	5/14 (35.7%)‡	7.20 ± 1.30	7.20 ± 1.30	0/36

[a]Data are expressed as means ± SD. *p< 0.0005, †p<0.0001, ‡p<0.00001 significantly different as compared to control values (Fisher's exact test).

§p<0.01, ‖p<0.005 significantly different as compared to control values (Student's *t* test).

DISCUSSION

The aim of the present study was to assess the adverse effects on fertility and reproduction of male mice after ingestion of NaF in their drinking water. The model employed in this work has been used previously by several investigators to assess the toxicity of different compounds on fertility and reproduction in laboratory animals.[5,15] The concentrations of NaF used in this study were chosen according to previous studies.[5] The drinking water route of exposure was chosen to mimic human exposure and to reflect the impact on fertility of the sustained blood levels of fluoride that would occur from water consumption throughout the day.

The results presented here demonstrate that exposure of adult male mice to NaF at concentrations of 100, 200 and 300 ppm in drinking water for a period of 4 weeks had no significant effect on their fertility. There was, however, a statistically significant increase in the number of embryo resorptions in females impregnated by these exposed males. The increase in the number of resorbed embryos may be attributed to an increase in peri-implantation mortality of fertilized ova. Furthermore, there was a statistically significant increase in relative weights of seminal vesicles for animals exposed to 200 ppm NaF but not to the other lower or higher concentrations. This change in the accessory gland weight might suggest an alteration in the pattern of testosterone secretion. A reduction in serum testosterone concentration has been reported in patients with fluorosis.[3]

A significant increase in the relative weights of preputial glands was also observed in animal groups administered NaF at 200 and 300 ppm for 4 weeks. This gland produces behavior-modifying pheromones which alter fighting and other behavior.[16] In contrast to these results, exposure of male mice to NaF for 10 weeks had no significant effects on weights of accessory sex glands. These results suggest that the increase in relative weights of these glands observed in the 4-week exposed groups was transient and is reversed with longer exposure to NaF.

The main finding of the present study was the significant reduction in fertility of male mice exposed to NaF for 10 weeks at the three concentrations used. This impairment in male fertility was dose dependent and increased as the concentration of NaF increased. Several other animal studies of the effects of sodium fluoride on reproduction and development have shown decreased fertility in most animal species studied. Abnormal spermatozoa were produced by male Swiss mice administered 8 mg/kg of fluoride by intraperitoneal injection for five consecutive days.[11] Another study conducted on mice reported that exposure to 500 or 1000 ppm NaF for 3 months resulted in clear damage to spermatogenesis.[17] Additionally, mice exposed to fluoride concentrations of 100 and 500 ppm in drinking water showed a lack of maturation and differentiation of spermatocytes and loss of spermatogenesis.[12] Impaired fertility has also been reported in mice administered NaF at 125, 250, and 500 ppm in their feed.[18] Furthermore, it has been reported that *in vitro* exposure of human sperm to

fluoride (250 mM) results in altered lysosomal activity, altered glutathione levels, and morphological anomalies, producing decline in sperm motility.[2] In rabbits, disruption and degeneration of seminiferous tubules were observed when NaF was orally administered at 10 mg/kg for 29 months.[19]

In contrast to the above findings, other work has reported that fluoride does not affect spermatogenesis or sperm morphology.[20] In a study using $B6C3F_1$ mice maintained on 75 ppm NaF in drinking water for 21 weeks, no effect on spermatogenesis was observed.[21]

In summary, our work has shown that sodium fluoride administered in drinking water at average doses of up to 39.19 mg/kg/day for 4 weeks in the mice had no significant effects on fertility of male mice. However, ingestion of sodium fluoride at doses of up to 27.25 mg/kg/day for 10 weeks did adversely affect their fertility.

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