

## REVERSIBLE FLUORIDE INDUCED FERTILITY IMPAIRMENT IN MALE MICE

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**SUMMARY:** Sodium fluoride (NaF) fed to adult male albino mice at a dose of 10 mg and 20 mg/kg body weight, caused a significant decrease in sperm count and motility. Scanning electron microscopy and silver nitrate staining showed large numbers of deflagellated spermatozoa, with acrosomal, midpiece and tail abnormalities. The treatment caused loss of fertility rate when normal cycling female mice were mated with treated males. Withdrawal of treatment for a period of 2 months resulted in a significant recovery in sperm count and sperm motility as well as in fertility rate.

**Key words:** Acrosomal integrity; Fertility impairment; Mice; NaF; Sperm count; Sperm motility.

### Introduction

The human population are exposed to fluoride from various sources such as soil, water and air. Extensive research has been carried out during the past several decades on skeletal and dental fluorosis (1). However, the effects of fluoride on the reproductive organs leading to loss of fertility is incomplete and conflicting. Tao and Suttie (2) reported that fluoride had no essential role in reproduction of female mice. On the contrary, Messer *et al.* (3,4) found that low fluoride intake by female mice impaired their fertility and reproductive capacity although growth rate and litter size were not affected.

Chinoy and Sequeira (5) reported that reproductive organs of male mice were affected by 10 and 20 mg/kg body weight of NaF ingested for 30 days. The testis, epididymides and vas deferens showed more alterations in their histology and histoarchitecture than seminal vesicle and prostate. However, all induced effects on the structure of reproductive organs were reversible after treatments were discontinued for two months. Therefore, it was clearly demonstrated that the effects induced by NaF treatment were transient and reversible and hence no permanent damage occurred. The present study was undertaken to investigate the effects of fluoride on mouse sperm structure and motility as well as fertility.

### Materials and Methods

Sodium fluoride (10 and 20 mg/kg body weight) was administered orally to 20 healthy Swiss strain adult mice (20-30 g) in each group. The animals were maintained on standard chow; water was given *ad libitum*. They were housed in an air conditioned animal house at a temperature of 26 ±2°C and exposed to 12 to 14 daylight hours. The animals were divided into a control group and four treatment groups (Table 1). After treatment for 30 days, NaF

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was withdrawn for one or two months. The male mice were autopsied by cervical dislocation and the percent sperm motility and sperm count of the cauda epididymis from control, treated and withdrawal groups of mice were determined by using Neubauer chamber of the Haemocytometer of the Prasad *et al* (6) method and expressed as percentage and millions/mL respectively.

To conduct the fertility test, normal cycling females were cohabited with treated males on the 31st day after treatment and in the withdrawal group at the end of one or two months respectively in the ratio of 2:1. The vaginal smear was checked the following morning to observe the presence of sperm which indicated that mating had occurred; this was day "0" of pregnancy. The females were separated from the males and allowed to remain on a normal diet for 16 days after which they were autopsied. The uteri were opened longitudinally and the number of implantation sites in each uterine horn as well as the number of corpora lutea in the ovary were counted according to WHO protocol MB-50 (7).

The acrosomal integrity of the sperm from the cauda epididymis of control, treated and withdrawal group of mice was studied using the modified silver nitrate technique (8). The methods of Chinoy and Sanjeevan (9) and Chinoy and Chinoy (10) were used for scanning electron microscopic observations of mouse cauda epididymal spermatozoa, under normal as well as treated conditions.

## Results

**Sperm Motility:** The cauda epididymis sperm motility decreased significantly ( $p < 0.001$ ) after 30 days treatment with both doses compared to control (Table 2). However, withdrawal of treatment for two months resulted in almost complete recovery (Table 2).

**Sperm Count:** The cauda epididymal sperm count of treated mice had decreased in comparison to the control (Table 2). After withdrawal of treatment, recovery was noted to have increased after two months (Table 2). In the uteri of females mated with treated males, the implantation sites were absent compared to 12-14 in the control, so that fertility was nil in treated mice (Table 2). Withdrawal of treatment resulted in significant recovery of fertility (Table 2).

Scanning Electron Microscopy (SEM) of normal cauda epididymal spermatozoa had scimitar shaped head (Figure 1). In the treated mice spermatozoa from cauda epididymis, head, midpiece and tail showed abnormalities compared to the control. Deflagellated spermatozoa were also observed (Figures 2 and 3).

Silver nitrate staining of cauda epididymal sperm of the control mouse revealed a clear differentiation of the acrosomal, post acrosomal and midpiece regions (Figure 4). However in NaF treated animals, the staining was diffuse with no proper demarcation (Figures 5 and 6).

## Discussion

Treatment induced a loss in fertility rate when normal cycling female mice were mated with treated males. Hall and Howell (11) observed that infertility is a relatively common manifestation of deficiency in trace elements, including deficiencies of copper, zinc, manganese, iodine and

selenium. Messer *et al* (3) demonstrated that low fluoride intake over two generations showed a progressive decline in litter production in female mice and addition of fluoride to the diet of females restored their reproductive capacity, even though earlier they were demonstrated as infertile. Kour and Singh (12) reported that in fluoride ingested mice testis showed a lack of maturation and differentiation of spermatocytes, cessation of spermatogenesis and necrotic seminiferous tubules. Moreover, a clear relationship between fluorosis and testis damage was observed by these authors in mice administered 500 and 1000 ppm NaF. Similar results have been obtained in mice given 10 and 20 mg NaF/kg body weight for 30 days (5). The testis, epididymis and vas deferens were affected more than seminal vesicles and prostate. As a consequence of the alterations in histology of these organs by NaF, particularly epididymis and vas deferens, their internal milieu is rendered

Table 1

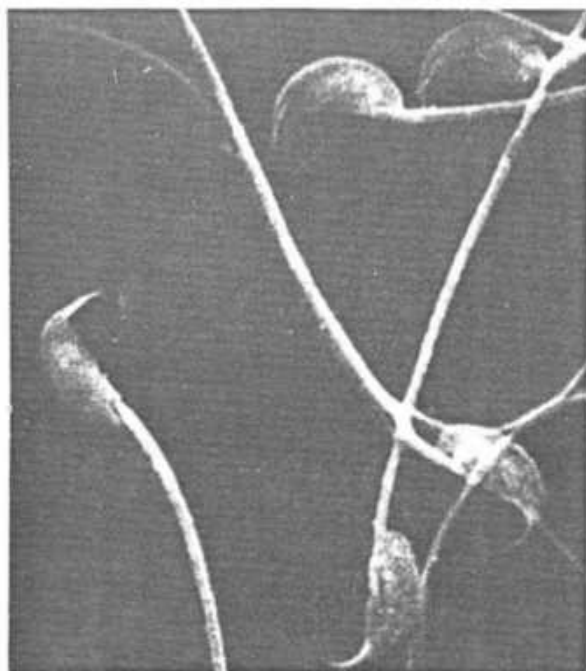
S1 No.	Group	Treatment	Duration (Days)	Day of Autopsy
1.	Control	—	—	Along with Treated
2.	NaF Treated	10 mg/kg Body Weight Equivalent to 230 ppm/ animal/day	30	31st
3.	NaF treated	20 mg/kg Body Weight Equivalent to 400 ppm/ animal/day	30	31st
4.	NaF treated	Withdrawal of Treatment for one month	30	31st
5.	NaF treated	Withdrawal of treatment for two months	60	61st

Table 2

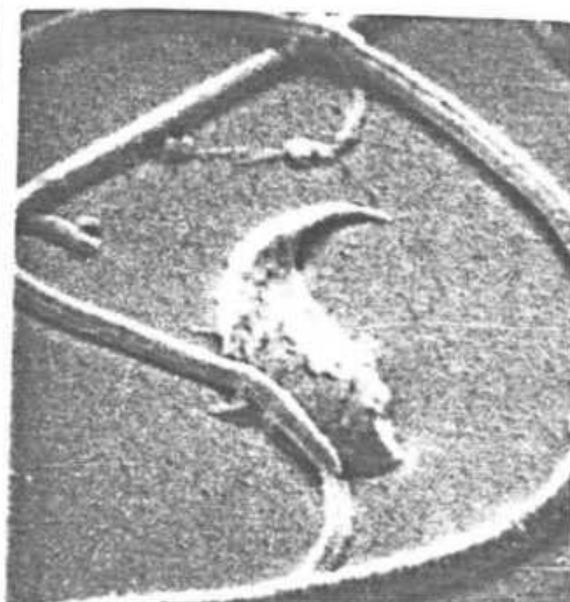
Sperm Motility, Count and Fertility Rate in Control, NaF-Treated and NaF-Withdrawal Group of Mice

Parameter	Control	NaF-Treated		NaF-Withdrawal	
		10 mg/kg body weight	20 mg/kg body weight	1 month	2 months
Cauda Epididymal Sperm motility (%)	80.0 ±0.28	38 ±2	24 ±1	44 ±0.3	74.6 ±1.7
Cauda Epididymal Sperm Count (10 <sup>6</sup> /mL)	45.0 ±0.58	34 ±3	32.8 ±0.5	37.7 ±0.6	43.4 ±1.6
Fertility Rate	95-100% +ve	nil	nil	nil	75% +ve

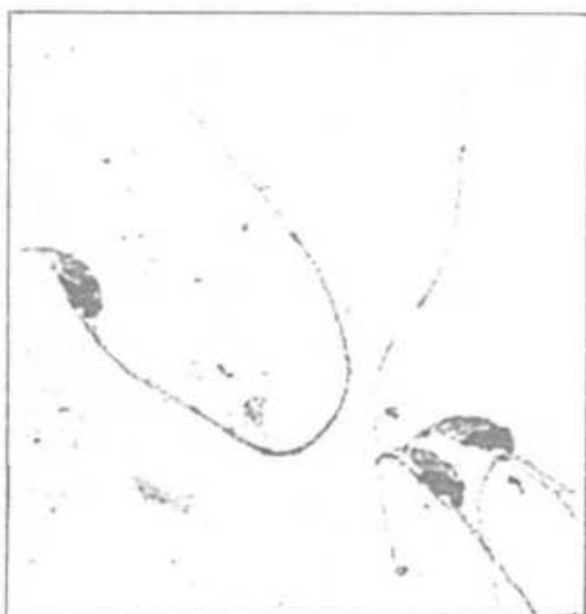
Figure 1 (right)  
Normal Spermatozoa



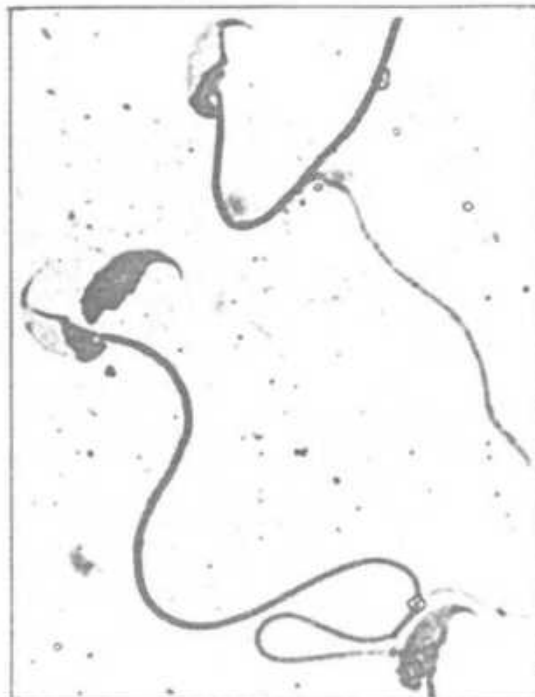
Figures 2 and 3 (below)  
Abnormal Spermatozoa



**Figure 4 (right)**  
Clear Demarcation (Control)



**Figures 5 and 6 (below)**  
Poor Demarcation (Treated)



hostile for the sperm motility, metabolism and survival (5), which was probably responsible for loss of fertility. Another factor leading to reduction of fertility might be due to the large number of deflagellated spermatozoa, as well as their acrosomal, midpiece and tail abnormalities caused by scanning electron microscopy and the modified silver nitrate staining technique (8).

The fluoride may interfere with spermatozoa maturation at the epididymal level or with the secretion of accessory glands. Since the histology of the reproductive organs was affected, their metabolism would be altered. The present study therefore elucidates certain important features of fluoride effects, namely (1) induction of infertility in male mice by altering the sperm structure and function; (2) the changes were transient and reversible since sperm density and motility were restored and significant fertility regained.

### Conclusion

NaF does not cause permanent damage to reproductive organs, since normalcy in histoarchitecture and fertilizability of spermatozoa was restored within 2 to 3 months after withdrawal of treatment.

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### References

- 1 Susheela AK (Ed). *Fluoride Toxicity*. ISFR, New Delhi 1985.
- 2 Tao S, Suttie JW. Evidence for a lack of an effect of dietary fluoride level on reproduction in mice. *Journal of Nutrition* 106 1115-22 1976.
- 3 Messer HH, Armstrong WD, Singer L. Fertility impairment in mice on a low fluoride intake. *Science* 177 893-4 1972.
- 4 Messer HH, Armstrong WD, Singer L. Influence of fluoride intake on reproduction in mice. *Journal of Nutrition* 103 1319-26 1973.
- 5 Chinoy NJ, Sequeira E. Effects of fluoride on histoarchitecture of reproductive organs of male mouse. *Reproductive Toxicology* 3 261-8 1989.
- 6 Prasad MRN, Chinoy NJ, Kadam KM. Changes in succinic dehydrogenase levels in rat epididymis under normal and altered physiologic conditions. *Fertility and Sterility* 23 186-90 1972.
- 7 *A Method for Examining the Effect of Plant Extract Administered Orally on the Fertility of Male Rats* (Protocol MB-50). World Health Organization, Geneva, 1983.
- 8 Chinoy NJ, Ranga GM, Highland HN et al. A modified method for the differential staining of spermatozoa using alcoholic acidic silver nitrate. *International Journal of Fertility* (in press) 1992.
- 9 Chinoy NJ, Sanjeevan AG. Effects of intraepididymal and intrascrotal copper device on rat spermatozoa. *International Journal of Andrology* 3 719-37 1980.
- 10 Chinoy NJ, Chinoy MR. Infertility induced by prostaglandins in the male albino rats by edrenergic block in the vas deferens. *International Journal of Fertility* 66 1-7 1981.
- 11 Hall GA, Howell JM. *British Journal of Nutrition* 23 41 1969. Cited by Messer et al 1972 (reference 3).
- 12 Kour K, Singh J. Histological findings of mice testes following fluoride ingestion. *Fluoride* 13 160-2 1980.