THE EFFECT OF SODIUM FLUORIDE INTOXICATION ON THE
ESTROUS CYCLE AND OVARIAN HORMONES IN RATS

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ABSTRACT: The objective of this study was to investigate the toxic effects of
different concentrations of sodium fluoride (0 [control], 5, 10, 15, and 20 mg NaF/kg
body weight/day) administered in drinking water for 30 days, on estrous cycle and
ovarian hormones of adult female albino rats (Rattus norvegicus). The rats in the two
higher dose groups (15 and 20 mg NaF/kg body weight/day) showed clinical signs of
toxicity unlike those exposed to NaF at a concentration of 5 mg NaF/kg body weight/
day. Body weight (bw) was significantly reduced in the rats ingesting 10 (p≤0.05), 15
(p≤0.001), and 20 (p≤0.001) mg NaF/kg bw/day and ovarian weight was significantly
reduced in the rats ingesting 15 (p≤0.05) and 20 (p≤0.001) mg NaF/kg bw/day. Vaginal
secretions from the 30 female rats were collected every morning for a month and the
changes in the estrous stages observed. The duration of the proestrous phase was
significantly increased in the 10 mg (p≤0.05), 15 (p≤0.001), and 20 mg (p≤0.001) NaF/
kg bw/day groups. In the 15 and 20 mg NaF/kg bw/day groups there were significant
decreases in the diestrous (p≤0.001), estrous (p≤0.05), and metaestrous (p≤0.05)
phases. The marked alteration in the estrous cycle was caused by decreased
hormonal concentrations of luteinising hormone (LH) (p≤0.05 in the 15 and 20 NaF/kg
bw/day groups), follicle-stimulating hormone (FSH) (p≤0.001 in the 10, 15, and 20 NaF/
kg bw/day groups), and estrogen (p≤0.05 in the 10 NaF/kg bw/day group; p≤0.001 in
the 15 and 20 NaF/kg bw/day groups). These hormones are responsible for ovulation.
The results indicate that exposure of female albino rats to NaF in drinking water might
have some immediate harmful effects on the reproductive system.

Keywords: Body weight; Estrous cycle; Follicle-stimulating hormone (FSH); Luteinising hormone (LH); Ovarian hormones; Ovarian weight; Rats (Rattus norvegicus).

INTRODUCTION

Prolonged exposure to hydrogen fluoride and the fluoride ion (F) through water,
air, and soil results in its accumulation in the body, predominantly in the teeth and
bones with dental mottling (dental fluorosis) and bone deformities (skeletal
fluorosis) in both man13 and domestic animals.4-8 Besides these ostearthly
abnormalities, nonskeletal changes due to chronic exposure to F have also been
observed including gastrointestinal disturbances, neurological disorders,
reproductive dysfunctions, apoptosis, excitotoxicity, genotoxicosis, and
teratogenic effects.9 An epidemiological study to assess whether F could affect
human birth rates using a US database of drinking water systems showed an
association between decreasing total fertility rate and increasing F levels.10 These
studies have suggested that F toxicity may cause adverse effects in the
reproductive system of males living in fluorosis endemic areas.11 A decreased
fertility rate due to sodium fluoride (NaF) toxicity has been found in the female rat
(Rattus norvegicus).12 Although there are a number of studies regarding the toxic
effects of NaF exposure on the male reproductive system in humans,13 rats,14,15

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and mice,\textsuperscript{16} studies on the female reproductive system are very limited. In female rats, estrous cycles are characterized by morphological changes in the ovaries, uterus, and vagina.\textsuperscript{17} The rat vagina can be considered to be an indicator of ovarian function and to reflect the activity of the sex hormones.\textsuperscript{18} Although estrous cycles are influenced by light, seasons of the year, and life circumstances, they occur without seasonal influence in rats submitted to environmental control under laboratory conditions.\textsuperscript{19} Thus, the present study was undertaken to investigate the effect in female albino rats of NaF exposure for 30 days on the estrous cycle, ovarian changes, and serum concentrations of the gonadotropin hormones, follicle-stimulating hormone (FSH), and luteinising hormone (LH).

**MATERIALS AND METHODS**

*Animals:* Thirty female albino rats (*Rattus norvegicus*) were selected for the study, weighing about 180–200 g. Each rat were caged separately, and raised in a ventilated animal house of the Department of Zoology, RTM Nagpur University, Nagpur, India, under the controlled temperature of 25±2°C on a 12 hr light/dark cycle. The animals were acclimatized for 7 days prior to the beginning of the study.

*Treatments:* The rats were randomly divided into 5 groups with each group consisting of 6 animals. The first group, I, served as a control and was provided with saline/defluoridated water while the remaining animals in groups II, III, IV, and V were treated with doses of NaF (Sigma chemical company, USA) at 5, 10, 15, and 20 mg NaF/kg bw/day, respectively, orally in their drinking water for 30 days (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Duration of exposure (days)</th>
<th>No. of animals</th>
<th>Day of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (control)</td>
<td>Saline / defluoridated water</td>
<td>30</td>
<td>6</td>
<td>31st</td>
</tr>
<tr>
<td>II</td>
<td>5 mg NaF/kg bw/day</td>
<td>30</td>
<td>6</td>
<td>31st</td>
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<tr>
<td>III</td>
<td>10 mg NaF/kg bw/day</td>
<td>30</td>
<td>6</td>
<td>31st</td>
</tr>
<tr>
<td>IV</td>
<td>15 mg NaF/kg bw/day</td>
<td>30</td>
<td>6</td>
<td>31st</td>
</tr>
<tr>
<td>V</td>
<td>20 mg NaF/kg bw/day</td>
<td>30</td>
<td>6</td>
<td>31st</td>
</tr>
</tbody>
</table>

The experimental protocol was approved by the Institutional Animal Ethics Committee (Register number 478/01/a CPCSEA) of the RTM Nagpur University, Nagpur, prior to the commencement of the study. At the end of the protocol the animals were sacrificed and the ovaries excised and weighed. Blood samples were collected by cardiac puncture for hormonal assessment.

*Assessment of estrous cycle:* During the 30 days of treatment, every morning between 9:00 to 10:00 am, vaginal secretions were collected with a plastic pipette filled with 10 µL of normal saline (0.9% NaCl) by inserting the tip into the vagina of the rats. One drop was collected with a clean tip from each rat and placed on a
glass slide. The slides were stained with methylene blue and observed under a light microscope with a 40× objective lens for evidence of diverse cells.

**Detection of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estrogen in the serum:** The blood samples from the different dose groups were centrifuged at 3,000 rpm for 10 min to obtain the serum. The serum samples were used for further analyses of the estrogen, LH, and FSH levels, by an ELISA Kit according to the manufacturer’s recommended instructions.

**RESULTS**

**Evaluation of body and ovarian weight:** Compared to the control group, the final body weight of the rats was significantly reduced in the 10 (p≤0.05), 15 (p≤0.001), and 20 (p≤0.001) mg NaF/kg bw/day groups. There was no significant body weight change in the 5 mg NaF/kg bw/day group (Figure 1).

![Figure 1. Body weights (g) of the control and NaF-treated rats. Values are expressed as mean±SEM (n=6 for each group). Comparing the initial and final body weights with the control group: *p≤0.05; †p≤0.001; where no symbol is shown no significant difference was present.](image)

As shown in Table 2, the right and left ovarian weights from the control and the NaF-treated animals showed no statistically significant difference at 5 and 10 mg NaF/kg bw/day but significant decreases were present in the 15 (p≤0.05) and the 20 (p≤0.001) mg NaF/kg bw/day treatment groups as compared to the control group.
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Evaluation of estrous stage in female rats: The cyclic changes observed in the vaginal smear provided a reasonable index of ovarian activity and the hormonal synthesis of estrogen and progesterone. Compared to the control group, the NaF-treated rats showed a trend towards reduced estrous cyclicity which increased as the NaF dose increased (Figure 2). Compared to the control group, the time in the proestrous phase was significantly increased in the 10 (p ≤ 0.05), 15 (p ≤ 0.001), and 20 (p ≤ 0.001) mg NaF/kg bw/day groups. Compared to the control group, the rats in the 15 and 20 mg NaF/kg bw/day groups had significant reductions in the diestrous (p ≤ 0.001), estrous (p ≤ 0.05), and metaestrous (p ≤ 0.05) phases.

Table 2. Ovarian weight (mg/100g body weight) of the treated and control groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment (mg NaF/kg body weight/day)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Right ovary weight</td>
<td>25.66±0.56</td>
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<tr>
<td>(mg/100g bw)</td>
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<tr>
<td>Left ovary weight</td>
<td>25.34±0.21</td>
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<tr>
<td>(mg/100g bw)</td>
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Values are expressed as mean±SEM (n=6 for each group). Compared with control: *p ≤ 0.05; †p ≤ 0.001; where no symbol or ns is shown no significant difference was present.

Figure 2. Effect of NaF on the estrous cycle of albino rats. Data represent the mean±SEM (n = 6 for each group). Compared to the control: *p ≤ 0.05; †p ≤ 0.001.
Three types of cells could be recognized in the methylene blue stained smears of the vaginal secretions: (i) epithelial cells which were round and nucleated, (ii) squamous cornified cells which were irregular in shape and without a nucleus, and (iii) leukocytes which were small round cells (Figures 3 a, b, c, and d).

Figures 3a, 3b, 3c, and 3d. Photomicrograph of vaginal smears from female rats at different phases of the estrous cycle in the control group (methylene blue stain, 40×). 3a: proestrus, the arrow points to a nucleated epithelial cell; 3b: estrus, the arrow points to a squamous cornified cell; 3c: metestrus, the upper arrow points to a squamous cornified cell, the lower arrow points to some leucocytes; 3d: diestrus, the arrow points to some leucocytes.

The ratios of the different cell types were used as an index marker for assessing the phases of the estrous cycle. In the NaF-treated rats, in which the alteration in the phases did not follow the sequence of proestrus, estrus, metaestrus, and diestrus (or intermediates), the cycles were considered to be irregular cycles (Figures 3 e, f, g, and h).
Estrogen level in the serum of female rats: Compared to the control group, the estrogen levels were significantly lower in the 10 (p ≤ 0.05), 15 (p ≤ 0.001), and 20 (p ≤ 0.001) mg NaF/kg bw/day groups (Figure 4). No significant difference was present in the 5 mg NaF/kg bw/day group compared to the control group.

Concentration of FSH and LH in the serum of female rats: Compared to the control group, the FSH levels were significantly lower (p ≤ 0.001) in the 10, 15, and 20 mg NaF/kg bw/day groups (Figure 5).
Compared to the control group, the LH levels were significantly lower ($p \leq 0.05$) in the 15 and 20 mg NaF/kg bw/day groups (Figure 6). No significant difference was present in the 5 and 10 mg NaF/kg bw/day groups compared to the control group.

**Figure 4.** Effect of different doses of NaF on the serum estrogen of female albino rats. Data represent the mean±SEM ($n = 6$ for each group). Compared to the control: $^*p \leq 0.05$; $^†p \leq 0.001$.

**Figure 5.** Effect of different doses of NaF on the serum FSH of female albino rats. Data represent the mean±SEM ($n = 6$ for each group). Compared to the control: $^†p \leq 0.001$. 
DISCUSSION

In the present investigation, a significant reduction was found in the body and ovarian weights of the NaF-treated rats as compared to the control group. Similar results have been reported in rats and mice and may be due to an adverse effect of F on metabolism or physiology.20-23

We found that the phases of the estrous cycle in the experimental groups showed a pattern of dose dependent changes, with a prolongation of the proestrus phases of the cycle in the 10, 15, and 20 mg NaF/kg bw/day groups, when compared with the control group, which is suggestive of an antifertility effect (Figure 2).24-26

During the estrous cycle, prolactin, LH, and FSH remain low until increasing in the afternoon of the proestrus phase.27 The estradiol levels begin to increase at the metestrus phase, reach peak levels during the proestrus phase, and return to the baseline at the estrous phase.27 Progesterone secretion increases during metestrus and diestrus and then decreases before rising again to reach a second peak towards the end of proestrus.27,28 The delay in the estrous cycle with NaF treatment is probably due to the NaF inhibiting the ovarian hormonal function, possibly through compromising cellular integrity and function.

The present study also revealed that the ovarian secretion of estrogen was significantly decreased in the groups treated with 10, 15, and 20 mg NaF/kg bw/day. It is accepted that the secretion of the reproductive hormones from the ovary is regulated by the release of LH and FSH from the anterior pituitary gland.29 We
found that both FSH and LH secretion were significantly reduced in the groups treated with 15 and 20 mg NaF/kg bw/day and that FSH secretion was also significantly reduced in the group treated with 10 mg NaF/kg bw/day. This marked decrease of LH and FSH secretion could explain the blockade of ovulation and the estrous cycle by the NaF treatment. Many investigators have demonstrated that the LH release surges at the proestrous stage are responsible for ovulation. The inhibition of this release by NaF could disrupt ovulation by decreasing the number of mature follicles or inducing an estrous cycle disruption at a rest stage. These results may point to one of the causes of the low fertility observed in the NaF-treated groups. More similar studies will help to further evaluate the aetiology of infertility in female animals with prolonged F exposure.

CONCLUSION

NaF may disrupt ovulation and the estrous cycle in rats by reducing LH and FSH secretion. Further similar research will help to further the understanding of the aetiology of infertility in female animals with prolonged F exposure.

ACKNOWLEDGEMENT

The authors wish to thank Dr SL Choubisa, Regional Editor for India for Fluoride, for his valuable suggestions.

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