EFFECTS OF HIGH FLUORIDE ON SPERM QUALITY AND TESTICULAR HISTOLOGY IN MALE RATS

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SUMMARY: Sixty-four forty-day old male Wistar rats were divided randomly into two groups of thirty-two each. With one group untreated as controls, the other group was administered 150 mg NaF/L (68 ppm F^-) in their drinking water to assess the effects of high fluoride on sperm quality and testicular histology at different developmental stages. In contrast to the control group, the F-treated rats exhibited a decline in sperm viability and a significant increase of sperm abnormalities 50, 80, 100, and 120 days after administration of sodium fluoride. Sperm density declined markedly at day 80 and day 120. The number of seminiferous epithelium cell layers (NSECL), the thickness of the seminiferous tubule (TST), and the diameter of the seminiferous tubule (DST) in the testis all decreased at day 50, 100, and 120. In short, the semen quality was impaired by fluoride in the drinking water, and the histological changes in the seminiferous epithelium of testicular tissues may be responsible for the diminished sperm quality in male rats.

Keywords: Fluoride and sperm; Male rats; Seminiferous epithelium histology; Sperm quality; Testicular pathology.

INTRODUCTION

It is well known that the effects of fluoride on biological tissues are often extensive. For some years, damage by fluoride to hard tissues and brain tissue of animals has been studied by our research group.[1-9] However, the effects of fluoride on reproductive functions are not fully understood, and reported findings are conflicting. Epidemiological investigations indicate that fluoride may cause adverse effects in the reproductive system of males living in fluorosis endemic areas.[10,11] On the other hand, Tao and Suttie[12] reported that fluoride does not play a disruptive role in mammalian reproduction. Moreover, laboratory studies by Collins et al[13] indicated that fluoride does not adversely affect spermatogenesis or endocrine function at 25, 100, 175 and 250 mg F^-/L in the drinking water of male rats. However, Cui et al.[14] found that 150 mg NaF/L (= 68 mg F^-/L) administered to male rats in their drinking water for 10 weeks caused a significant decrease in their sperm count and sperm mobility. Moreover, Chinoy et al.[15] found that fluoride causes diminished sperm quality and lowered reproductive hormone levels in their animal experiments.

In the present study, changes were examined in sperm quality and testicular morphological parameters in male rats at different periods after exposure to elevated fluoride in the drinking water.

MATERIALS AND METHODS

Materials: For this study, standard diets and forty-day old male Wistar rats (each weighing approximately 50 g) were obtained from the Experimental Animal Center of Shanxi Medical University.

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Establishment of animal model: Sixty-four of the above male rats were divided randomly into two groups of thirty-two: a control group, which was given distilled water, and a high fluoride group to which 150 mg NaF/L (= 68 mg F^-/L) was administered in their drinking water. All rats were maintained on normal standard diets under standard temperature (22–25ºC), ventilation, and hygienic conditions. At day 50, 80, 100, and 120, eight rats from each group were randomly selected, weighed, and injected with 20% urethane solution for fatal anaesthesia. Blood was collected from the eyeball for separating serum, and the testes and epididymides tissues were carefully removed and blotted free of blood for further study.

Evaluation of sperm quality: The cauda epididymal sperm suspension was prepared in normal saline at 37ºC. Sperm viability (live/dead ratio) and sperm density in all rats were calculated by the method of Prasad et al.[16] and expressed as percentage viability and $\times 10^{10}$/L, respectively. The percentage of abnormal sperm was scored in 10 to 20 separate fields using 1% trypan blue by the method of Talbot and Chacon.[17]

Morphology of testis tissue: The testes were fixed in Karnory substrate[18] for 24 hr and subsequently embedded in paraffin. Sections six-micrometers thick were processed and stained with hematoxylin and observed under a light microscope. The number of seminiferous epithelial cell layers (NSECL), the thickness of seminiferous tubules (TST), and the interior diameter of the seminiferous tubules (DST) in the testis section were determined by Image-Pro® Plus Version 5.1 micrograph analysis software (Media Cybernetics Inc., USA). Two sections were prepared from the testes of each rat, and five seminiferous tubules were examined randomly in each histological section.

RESULTS

Changes in sperm quality: Sperm viability, density, and abnormality percentages in the cauda epididymis of the male rats at day 50, 80, 100, and 120 are shown in Tables 1, 2, and 3, respectively.

Table 1. Change in sperm viability (%) in male rats (mean ± S.E.)

Day	No. of animals in each group	Control	High fluoride
50	8	92.75±1.11	74.00±0.91†
80	8	90.50±0.65	74.00±2.04†
100	8	84.50±0.65	61.50±1.33†
120	8	82.25±0.85	57.25±2.17†

†P<0.01 (compared with the control group in all tables).

Table 2. Sperm density (%) in male rats (mean ±S.E.)

Day	No. of animals in each group	Control	High fluoride
50	8	0.52±0.02	0.50±0.00
80	8	0.77±0.02	0.65±0.02*
100	8	1.56±0.02	1.39±0.05
120	8	3.28±0.02	2.61±0.08*

*P<0.05.

Table 3. Sperm abnormality ratio (%) in male rats (mean ± S.E.)

Day	No. of animals in each group	Control	High fluoride
50	8	6.25±0.48	15.50±1.32†
80	8	11.50±0.65	21.50±2.90†
100	8	11.75±0.48	28.75±1.49†
120	8	19.00±0.92	36.25±2.06†

†P<0.01.

Morphology of testis tissue: The testicular seminiferous epithelium morphology parameters are recorded in Table 4. Correlation coefficients (r) from the data are: 0.683 ($p<0.01$) between sperm viability and the number of seminiferous epithelium cell layers (NSECL); 0.846 ($p<0.01$) between sperm viability and the thickness of seminiferous tubules (TST); 0.429 ($p<0.05$) between sperm viability and the diameter of seminiferous tubules (DST); –0.588 ($p<0.01$) between abnormal sperm ratio and NSECL; and –0.385 ($p<0.05$) between abnormal sperm ratio and TST.

Table 4. Testicular seminiferous epithelium morphology parameters in male rats (mean ±S.E.)

Day	Group	No. of seminiferous tubules	No. of seminiferous epithelium cell layers (NSECL)	Thickness of seminiferous tubules (μm) (TST)	Diameter of seminiferous tubules (μm) (DST)
50	Control	80	6.6±0.2	160.30±4.75	265.65±3.40
	High fluoride	80	5.6±0.3†	138.86±7.47†	210.91±9.77†
80	Control	80	7.3±0.2	183.05±2.15	276.50±2.66
	High fluoride	80	7.1±0.3	178.85±3.24	267.05±2.66
100	Control	80	5.7±0.2	179.90±1.50	276.70±2.54
	High fluoride	80	4.8±0.2†	148.40±2.77†	260.40±3.39*
120	Control	80	5.7±0.2	180.85±2.12	276.90±2.21
	High fluoride	80	5.2±0.1†	150.50±2.15†	249.20±3.13†

*P<0.05; †P<0.01.

DISCUSSION

Effects of fluoride on sperm quality: Sperm quality is one of the important indexes of male reproductive function. Changes in sperm quality induced by fluoride have been demonstrated *in vivo* and *in vitro* in many species, including the rat, mouse, rabbit, gerbil, guinea pig, bank vole, chicken, and even people, from many studies in eleven countries.[10] However, experimental results differ. Some reports indicate that sodium fluoride does not affect sperm quality in rats,[13,19] whereas other experimental studies suggest that fluoride can cause low sperm quality and diminished fertility.[14,20,21] In the present study, a decline in sperm viability and a significant increase in sperm abnormality were observed at days 50, 80, 100, and 120 in male rats administered 68 mg of fluoride ion/L in their drinking water beginning when 40 days old. At days 80 and 120 the sperm density declined markedly. From these changes, we conclude that this concentration of fluoride in the drinking water of young rats impairs sperm quality, in agreement with the findings already cited.[14,20,21] Different experimental procedures

including how, how much, and how long fluoride was administered may account for some of these contradictory results.

Effects of fluoride on testicular morphology: In recent years, histological changes in reproductive organs of rabbits, rats, and mice induced by fluoride have been reported by many researchers.[10] In our studies we also found that NaF caused disorganization, denudation, and reduction in germinal epithelial cells of the seminiferous tubules and an accompanying absence of sperm in the lumina in histological sections. These histopathological changes are in accord with the results of Cui et al.[14] and of Chinoy et al.[22,23] It should be noted, however, that most of these reports refer to qualitative descriptions and reflect only the end stage of intoxication. In the present study, our dynamic observations and quantitative analysis showed that these characteristic changes occurred at various stages and not at all experimental periods. Thus the number of seminiferous epithelium cell layers (NSECL), the thickness of seminiferous tubules (TST), and the diameter of seminiferous tubule (DST) were significantly reduced in the high fluoride group at day 50, 100, and 120 in male rats. Nevertheless, at day 50 the distinct pigmentation of the spermatogenic cells was observed on the basis of histological sections, thereby indicating that spermatogonial function in the testicular tissue is developing at that point. At day 80, although the NSECL, TST, and DST do not show significant change in the testis tissue, loosening of the seminiferous epithelium cell walls and the lack of spermatozoa in the lumen can be distinctly observed. Both the reduction of the NSECL, TST, and DST and the histopathological changes are evident beginning at days 100 and 120. Our results thus show that damage by fluoride to testicular tissues changes with the length of toxic exposure.

Relationship between sperm quality and testicular morphology: It is known that histopathological changes in the testes are the histological basis of changes in spermatogenesis function, especially in the seminiferous tubule. Furthermore, normal testicular structure and the maintenance of its internal microenvironment are important for sperm production and for maintaining good quality. In this study, exposure to NaF caused a decline in sperm quality and a change of testicular morphological parameters in male rats at different days of exposure. This result might be connected with histopathological changes in the seminiferous tubules in the testes. Actually, the seminiferous tubule consists of the germinal epithelium and the peritubular tissue (lamina propria), [24] and the germinal epithelium consists of cells that include different developmental stages of germ cells.[25] Therefore, as morphological parameters of the seminiferous tubule, the changes of NSECL, TST, and DST in testicular tissues are closely associated with the observed decrease in the sperm quality. At present the exact relationship between changes in testicular morphological parameters and sperm viability, sperm density, and sperm abnormality ratio, respectively, are not known and clearly deserve further study.

In conclusion, under the conditions of these experiments, fluoride was found to decrease the sperm quality of male rats, with changes in the morphological parameters of the seminiferous tubule apparently being one of the pathways that lead to this decrease.

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