DECREASED SPERM HYPERACTIVATION AND LOW CATSPER1 EXPRESSION IN MICE EXPOSED TO FLUORIDE

Zilong Sun,[a] Bin Wang,[a] Ruiyan Niu,[a] Jianhai Zhang,[a] Jundong Wang[a]

Shanxi, China

SUMMARY: Sperm quality, sperm hyperactivation, and the gene expression of Catsper1 were studied in adult male Kunming mice exposed to 150 mg NaF/L for 7 weeks in their drinking water. Compared with the controls, sperm quality and the proportion of hyperactivated sperm were significantly decreased in mice treated with F. Likewise, the Catsper1 gene expression level was also significantly reduced in the treatment group. On the basis of these findings, we propose that low Catsper1 gene expression in sperm may be associated with decreased sperm hyperactivation by NaF.

Keywords: Catsper1; Male mice; Sperm hyperactivation; Sperm motility.

INTRODUCTION

Besides immunotoxic, neurotoxic, and cytotoxic effects induced by fluoride (F), the adverse influence of F on the reproductive system has become a major concern in many countries. The importance of reproductive health to offspring developments has prompted epidemiological investigations of the apparent connection between excessive F exposure to male infertility and low birth rates.[1,2] These findings are supported by numerous animal experimental studies that have demonstrated a negative impact of F ingestion on spermatogenesis,[3] sperm morphology and motility,[4-7] sperm capacitation,[8] and sperm fertilizing ability.[9-11]

Sperm hyperactivation is a kind of sperm movement pattern that occurs at or near the site of fertilization.[12,13] Because it is necessary for successful fertilization, sperm hyperactivation is an important index for evaluating the quality of mature sperm in mammals.

At present, however, little is known about toxic effects of F on sperm hyperactivation. Therefore, the purpose of this study was to assess the action of F on sperm hyperactivation to aid in gaining a better understanding of how F induces male infertility. To address this issue, we further observed the gene expression change of Catsper1, which is involved in the Ca^{2+} signaling pathway during hyperactivation process.[14, 15]

MATERIALS AND METHODS

Establishment of animal model: Fifty adult male Kunming mice, about 20 g in weight, were obtained from the Laboratory Animal Center, Institute of Zoology, Chinese Academy of Sciences, along with supplies of their standard diet. The mice were divided randomly into two groups of 25 each: a control group, which was given distilled water, and a treatment group, which was received 150 mg NaF/L in their drinking water. All mice had free access to food and water under standard

[a]For Correspondence: Prof Jundong Wang, Shanxi Key Laboratory of Ecological Animal Science and Environmental Medicine, Shanxi Agricultural University, Taigu, Shanxi, 030801, PR of China; E-mail: wangjd@sxau.edu.cn

temperature (22–25ºC), 12/12-hr light/dark cycle, ventilation, and hygienic conditions. The Institutional Animal Care and Use Committee of China approved the design of the study.

Sperm sample preparation and sperm quality evaluation: Seven weeks after establishing the animal model, all mice were sacrificed by cervical dislocation. Sperm from the cauda epididymis and vas deferens was allowed to disperse into human tubal fluid (HTF) medium at 37ºC. Aliquots of sperm suspensions were prepared for determination of sperm quality by the method of Huang et al.[5]

The rest of the sperm then were washed once with HTF medium by centrifugation with 200×g for 5 min, followed by incubation at 37ºC under 5% CO_2 for 30 min. After swim-up, supernatant containing motile sperm, was collected, and adjusted with HTF at a final concentration of 5×10^5 sperm/mL and then incubated for 1 hr at 37ºC under 5% CO_2 for hyperactivation analysis.

Assessment of hyperactivation: Sperm hyperactivation was measured as previously described by Ishijima *et al*.[16] Briefly, a 60-µL aliquot of sperm suspension was loaded on a 20-µm deep observation chamber fixed on slide which was pre-warmed at 37ºC, and covered with a glass coverslip. The motilities of sperm were observed with an Olympus BX51 microscope equipped with CCD DP70 video camera (Olympus Optical, Tokyo, Japan). At least 200 motile sperm per assay were counted, and the proportion of those exhibiting hyperactivated motility was determined. Immotile sperm were not included in the final analysis.

Sperm RNA extraction and quantitative real-time polymerase chain reaction (QRT-PCR): After hyperactivation evaluation, the sperm pooled from 5 mice were washed twice in PBS, and then resuspended in a somatic cell lysis buffer (0.1% SDS, 0.5% Triton X-100 in distilled H_2O). This procedure produced essentially pure sperm.[17] Total RNA in the sperm was extracted according to the Trizol (Invitrogen, USA) manufacturer's instructions.

Specific primers of Catsper1 and β-actin (Table 1) were designed with Primer 3.0 plus. QRT-PCR was conducted by using the Mx3000P™ QRT-PCR system (Stratagene, USA) and two-Step SYBR® QRT-PCR kit (Takara, China). Thermocycling conditions were as follows: after initial denaturation at 95ºC for 15 sec, 55 PCR cycles were started with thermocycling conditions at 95ºC for 5 sec, 61ºC for 20 sec, and 72ºC for 6 sec, and then followed by the reaction melting curve analysis to verify the specificity of the amplified products. β-Actin was used as an endogenous control to reflect the relative abundance of the target gene, which was obtained by calculating the ratio of the threshold cycle (Ct) values using following formula: Ct value of β-actin/Ct value of target gene.[18]

Table 1. Primer sequences with their corresponding PCR product size and position

Gene	Primers (5'→ 3')	Product location	Product (bp)	Gene bank Accession No.
β-actin	TTGCTGACAGGATGCAGAAG ACATCTGCTGGAAGGTGGAC	1005-1145	141	NM_007393.3
Catsper1	TAGACCCAAGCCATCAATCC CCATGCTGTTGGTATGGTTG	56-182	127	NM_139301.2

Data Analysis: Experimental data were expressed as mean values±SEM. With the help of SPSS 11.5 statistical software, differences between groups were evaluated by independent-samples t-test. If the P value was<0.05, the difference was considered to be statistically significant.

RESULTS

Effects of NaF on sperm quality: As shown in Table 2, NaF exposure brought about significant declines in sperm count, sperm motility, and sperm survival ($p<0.05$) in mice as compared to the controls.

Table 2. Sperm quality in male mice after 7 weeks of exposure to distilled water (Control group) or 150 mg NaF/L in their drinking water (Treatment group) (n=8; mean±SEM)

Groups	Weight(g)	Sperm count(10^6/ml)	Sperm motility(%)	Sperm survival(%)
Control	40.12±1.10	10.75±1.33	72.78±1.46	74.91±2.99
Treatment	40.85±1.68	6.60±0.80*	59.16±2.21*	62.04±4.37*

*p<0.05 (compared with the control group).

Effects of NaF on sperm hyperactivation: Photographs of the non-hyperactivated sperm (A) and the hyperactivated sperm (B) are shown in Figure 1. From Figure 2 it can be seen that a significant ($p<0.05$) decrease was observed in the proportion of hyperactivated sperm in mice administrated 150 mg NaF/L in their drinking water.

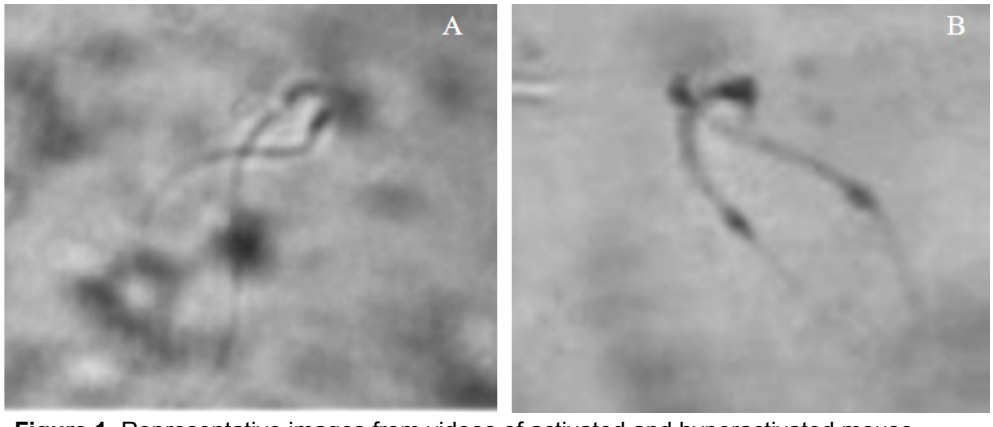

Figure 1. Representative images from videos of activated and hyperactivated mouse sperm. A) Activated sperm exhibiting symmetrical flagellar beating, B) Hyperactivated sperm displaying high amplitude and asymmetrical flagellar movement. Each image was produced from two actions of the same sperm by two flashes of a stroboscope, spaced 1/60 sec apart.

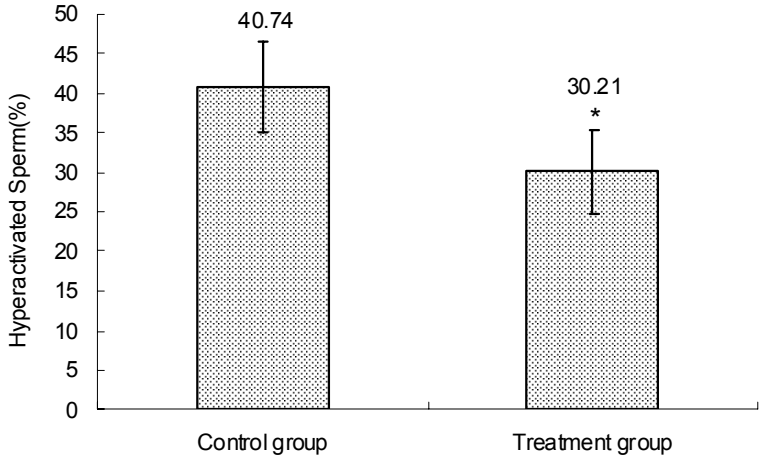

Figure 2. The proportion of hyperactivated sperm in mice from control and treatment groups. Values are expressed as mean±SEM (n=8). Bar labeled with asterisk indicates significant differences between treatments ($p<0.05$).

Effects of NaF on Catsper1 mRNA expression: Figure 3 presented the amplification plots for Catsper1 and β-actin genes in sperm of mice, and the original Ct values of these two genes in control and treatment groups

.

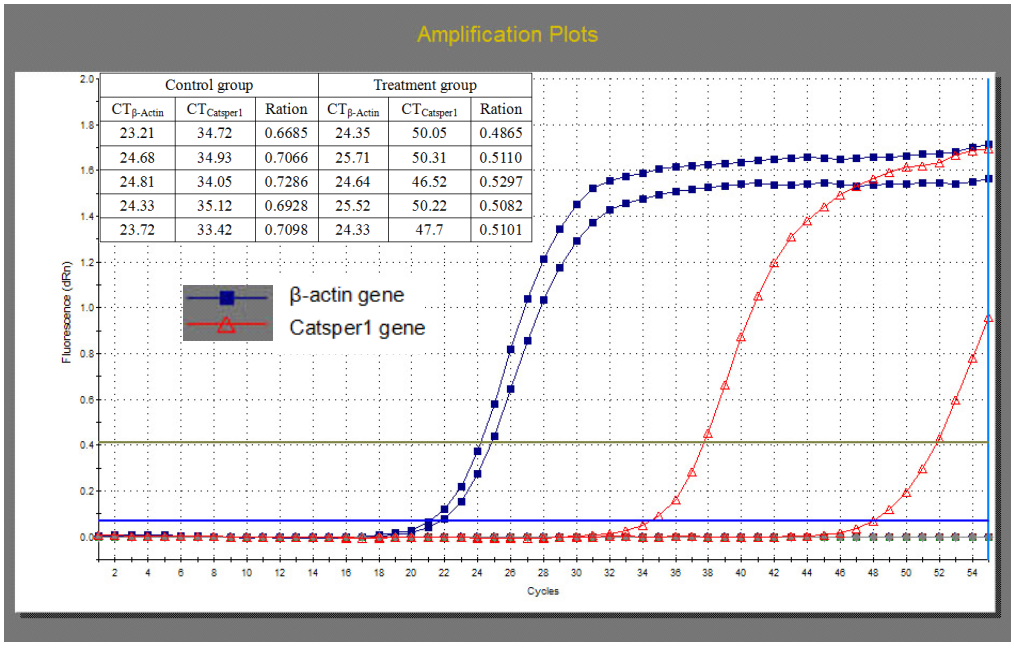

Figure 3. Amplification plots for Catsper1 and β-actin genes in sperm of mice in control and treatment groups, and the original Ct values of these two genes. Table on the left top shows original data of CT value and ration, of which ration refers to Ct value of β-actin/ Ct value of Catsper1.

As seen in Figure 4, compared to the control group, the gene expression of Catsper1 was significantly decreased by 27.4% in treatment group.

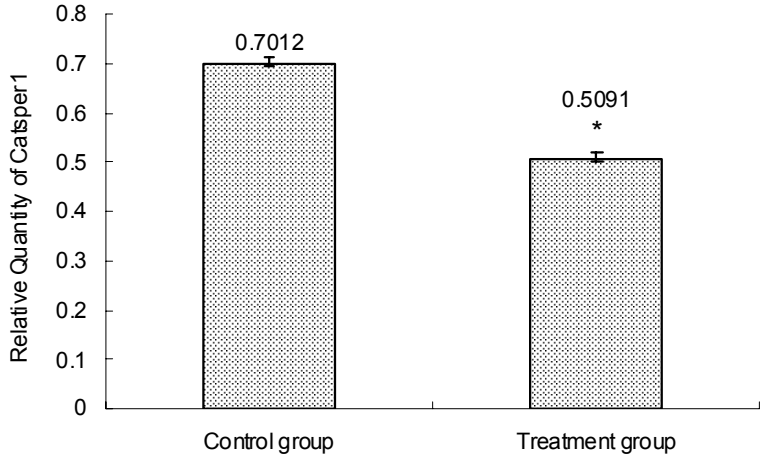

Figure 4. The relative expression levels of Catsper1 in sperm of mice in control and treatment groups. Values are expressed as mean±SEM (n=5). Bar labeled with asterisk indicates significant differences between treatments (P<0.05).

DISCUSSION

As described in our previous reports,[5-7,19] the sperm count of mice was decreased by treatment with NaF. F exposure also brought about a marked decline in the motility and survival of sperm.[20-22] The decrease in these three sperm properties indicated a low sperm quality which may correlate with male infertility. F is known to adversely affect the reproductive system of animals and human being. In the present study, our results are in agreement with these findings. However, conventional parameters, including sperm concentration, motility, and survival, are poor in predicting future fertility.[23] To better understand the toxic effects of F on reproduction, we felt it necessary to focus on alteration of sperm hyperactivation induced by F.

The movement of hyperactivated sperm is different from the normal activated sperm. It involves an increase in flagellar beat amplitude and bent asymmetry to enhance the ability of sperm to detach from the wall of the oviduct, to move around in the labyrinthine lumen of the oviduct, to penetrate mucous substances and, finally, to penetrate the zona pellucida of the oocyte.[12, 24, 25] However, very little is known about the toxic effects of F on sperm hyperactivation. Here, our result demonstrated, for the first time, that F inhibited the hyperactivated motility of sperm in mice, indicating that less sperm can meet requirements for fertilization. This finding could be a good basis to explore the cause of low birth rates in high F areas and in animals treated with F.

Although no specific signals has been identified for regulating hyperactivation occurrence, it is clear that an elevation of intracellular Ca^{2+} concentration correlates with the acquisition of hyperactivated motility, suggesting that ion channels are involved in this process.[16] Over the past decade, studies using gene

knockout mice have confirmed that Catsper is the putative Ca^{2+} channel essential for hyperactivation and also that male mice deficient Catsper1 gene were completely infertile.[14,26] In the present study, compared with the controls, the expression of Catsper1 gene in the F treated group was significantly decreased, which was corroborated by the decrease in hyperactivated sperm in the treatment mice.

Taking the above together, it is clear that F adversely affected sperm motility and hyperactivation in mice, probably through alteration of the Catsper1 gene. These changes induced by F may therefore be one of the reasons for low male fertility associated with increased exposure to F.

ACKNOWLEDGEMENTS

This research was sponsored by the China National Natural Science Foundation (Grant No. 30871899 and 30671545), the Program for Ministry of Agriculture (Grant No. 2009-Z47), the Shanxi Province Science and Technology Bureau Program (Grant No. 20090311036), and the Shanxi Province Natural Science Foundation (Grant No. 2009021035-1).

REFERENCES

1 Ortiz-Pérez D, Rodríguez-Martínez M, Martínez F, Borja-Aburto VH, Castelo J, Grimaldo JI, et al. Fluoride-induced disruption of reproductive hormones in men. Environ Res 2003;93(1):20-30.
2 Freni SC. Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. J Toxicol Environ Health 1994;42(1):109-21.
3 Pushpalatha T, Srinivas M, Sreenivasula Reddy P. Exposure to high fluoride concentration in drinking water will affect spermatogenesis and steroidogenesis in male albino rats. Biometals 2005;18(3):207-12.
4 Kumar A, Susheela AK. Ultrastructural studies of spermiogenesis in rabbit exposed to chronic fluoride toxicity. Int J Fertil Menopausal Stud 1994;39(3):164-71.
5 Huang C, Niu RY, Wang JD. Toxic effects of sodium fluoride on reproductive function in male mice. Fluoride, 2007;40(3):162-8.
6 Wan SX, Zhang JH, Wang JD. Effects of high fluoride on sperm quality and testicular histology in male rats. Fluoride 2006;39(1):17-21.
7 Zhang JH, Liang C, Ma JJ, Niu RY, Wang JD. Effects of sodium fluoride and sulfur dioxide on sperm motility and serum testosterone in male rats. Fluoride 2006;39(2):126-31.
8 Dvoráková-Hortová K, Sandera M, Jursová M, Vasinová J, Peknicová J. The influence of fluorides on mouse sperm capacitation. Anim Reprod Sci 2008;108(1-2):157-70.
9 Elbetieha A, Darmani H, Al-Hiyasat AS. Fertility effects of sodium fluoride in male mice. Fluoride 2000;33(3):128-34.
10 Chinoy NJ, Sharma A. Reversal of fluoride-induced alteration in cauda epididymal spermatozoa and fertility impairment in male mice. Environ Sci 2000;7(1):29-38.
11 Izquierdo-Vega JA, Sánchez-Gutiérrez M, Del Razo LM. Decreased in vitro fertility in male rats exposed to fluoride-induced oxidative stress damage and mitochondrial transmembrane potential loss. Toxicol Appl Pharmacol 2008;230(3):352-7.
12 Ho HC, Suarez SS. Hyperactivation of mammalian sperm: function and regulation. Reproduction 2001;122(4):519-26.
13 Stauss CR, Votta TJ, Suarez SS. Sperm motility hyperactivation facilitates penetration of the hamster zona pellucida. Biol Reprod 1995;53(6):1280-5.
14 Carlson AE, Westenbroek RE, Quill T, Ren D, Clapham DE, Hille B, et al. CatSper1 required for evoked Ca^{2+} entry and control of flagellar function in sperm. Proc Natl Acad Sci U S A 2003;100(25):14864-8.

15 Ren D, Navarro B, Perez G, Jackson AC, Hsu S, Shi Q, et al. A sperm ion channel required for sperm motility and male fertility. Nature 2001;413(6856):603-9.
16 Ishijima S, Mohri H, Overstreet JW, Yudin AI. Hyperactivation of monkey spermatozoa is triggered by Ca^{2+} and completed by cAMP. Mol Reprod Dev 2006;73(9):1129-39.
17 Ostermeier GC, Dix DJ, Miller D, Khatri P, Krawetz SA. Spermatozoal RNA profiles of normal fertile men. Lancet 2002;360 (9335): 772-7.
18 Wang H, Zhou Z, Xu M, Li J, Xiao J, Xu ZY, et al. A spermatogenesis-related gene expression profile in human spermatozoa and its potential clinical applications. J Mol Med 2004;82 (5): 317-24.
19 Liu HT, Niu RY, Wang JM, He Y, Wang JD. Changes caused by fluoride and lead in energy metabolic enzyme activities in the reproductive system of male offspring rats. Fluoride 2008;41(3):184-91.
20 Chinoy NJ, Mehta D, Jhala DD. Effects of fluoride ingestion with protein deficient or protein enriched diets on sperm function of mice. Fluoride 2006;39(1):11-6.
21 Chinoy NJ, Reddy VVPC, Mathews M. Beneficial effects of ascorbic acid and calcium on reproductive functions of fluoride treated prepubertal male rats. Fluoride 1994;27(2):67-75.
22 Chinoy NJ, Sequeira E. Reversible fluoride induced fertility impairment in male mice. Fluoride 1992;25(2):71-6.
23 Lewis SE. Is sperm evaluation useful in predicting human fertility? Reproduction 2007;134(1):31-40.
24 Bastiaan H, Franken D. The influence of homogenous zona pellucida on human spermatozoa hyperactivation, acrosome reaction and zona binding. Andrologia 2007;39 (1):7-11
25 Mortimer ST, Swan MA, Mortimer D. Effect of seminal plasma on capacitation and hyperactivation in human spermatozoa. Hum Reprod 1998;13(8):2139-46.
26 Xia JS, Reigada D, Mitchell CH, Ren DJ. CATSPER channel-mediated Ca^{2+} entry into mouse sperm triggers a tail-to-head propagation. Biol Reprod 2007;77(3):551-9.