

# Sperm Quality Parameters and Oxidative Stress: Exploring Correlation in Fluoride-Intoxicated Rats

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ABSTRACT

**Background:** Assessment of male fertility needs evaluation of sperm quality parameters, namely sperm count, viability, motility and morphology. **Aims:** The present study aimed to analyse and correlate oxidative stress with sperm quality parameters. **Settings and Design:** The male Wistar albino rats, weighing between 100 and 150 g, were employed in the present study under the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines with ethical clearance from the Institutional Ethical Committee. These rats were categorised into four groups with six rats in each as control and test animals. **Materials and Methods:** Young male Wistar albino rats, weighing between 100 and 150 g, were divided into four groups of six rats each. The first group of rats served as control ( $n = 6$ ) and was maintained under normal laboratory condition and was provided with clean drinking water, whereas rats in the second ( $n = 6$ ), third ( $n = 6$ ) and fourth ( $n = 6$ ) groups were orally intubated with sodium fluoride of 100 ppm, 200 ppm and 300 ppm, respectively, for 40 days. **Statistical Analysis Used:** After the treatment period of 40 days, animals were sacrificed and alterations in sperm quality parameters were analysed by complete randomised design SAS 9.4 and Statistical Package for the Social Sciences (SPSS) IBM 17 and judged significant if  $P < 0.05$ . **Results:** In the experiment, a negative correlation emerged between sperm motility, viability, count versus malondialdehyde (MDA) levels, whereas the level of MDA has a positive correlation with sperm abnormalities. Sperm motility, viability and count were positively correlated with activities of superoxide dismutase and catalase, whereas decreased activities of antioxidants were related to increased sperm morphological abnormalities. **Conclusion:** These results suggest that MDA causes a decline in sperm motility, count and viability and an increase in morphological abnormalities via oxidative damage of membrane lipids.

**KEYWORDS:** Antioxidant enzymes, fluoride, lipid peroxidation, oxidative stress, sperm count, sperm motility, sperm viability, spermatozoon

## INTRODUCTION

A close association has been found between deteriorating human health and elevated levels of fluoride in drinking water. Ingested fluoride is readily absorbed and distributed through blood and enters the lumen of the seminiferous tubule through the cytoplasm of epithelial cells of the epididymis.<sup>[1]</sup>

Elevated fluoride levels decrease the capacitation and acrosome reactions of spermatozoa and block the calcium signalling pathways. It is also involved in sperm hyperactivation, delays the maturation, differentiation of spermatocytes and disturbs the

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production and release of male sex hormones such as testosterone, estradiol and thyroid.<sup>[2]</sup> Ingestion of excessive fluoride also decreases the expression of epidermal growth factors and reduces the diameter of seminiferous tubules, which leads to disorganisation in the germinal epithelial cell layer.<sup>[3]</sup>

Sperm quality parameters and oxidative status are commonly assessed to estimate male fertility.<sup>[4-6]</sup> Nonetheless, studies concerning the simultaneous evaluation of these parameters are still scarce<sup>[7,8]</sup> and, in particular, those aimed to correlate these parameters with each other.<sup>[9-12]</sup> This study aimed to simultaneously evaluate the correlation between the oxidative status and sperm quality parameters. Associations between these parameters may provide valuable information on new potential markers for sperm quality assessment.

## SUBJECTS AND METHODS

### Animals

Young male Wistar albino rats, weighing between 100 and 150 g, were housed in polypropylene cages with stainless steel grill tops and fed with a standard rat pellet diet (Hindustan Lever Limited, India). The water was given *ad libitum*. The experiments were performed under the approval of the Animal Ethical Committee of Punjabi University, Patiala (Animal maintenance and Registration No. 107/99/Committee for the Purpose of Control and Supervision of Experiments on Animals-2012-11). All the procedures in the experiments were performed in accordance with the ethical standards of the Committee for the Purpose of Experiments on Animals, India.

### Experimental design

Young male Wistar albino rats, weighing between 100 and 150 g, were divided into four groups of six rats each. The first group of rats served as control ( $n = 6$ ) and was maintained under normal laboratory condition and was provided with clean drinking water, whereas rats in the second ( $n = 6$ ), third ( $n = 6$ ) and fourth ( $n = 6$ ) groups were orally intubated with sodium fluoride (NaF) of 100 ppm, 200 ppm and 300 ppm, respectively, for 40 days. After the treatment period of 40 days, animals were sacrificed and alterations in male reproductive system were analysed. At autopsy, the testes and epididymis were removed and cleared of the adhering tissues and weighed. The percent change in body weight compared to initial body weight and relative organ weight (weight per 100 g body weight) was computed. The testis and epididymis were used for total sperm count, motility, abnormal sperm counts and biochemical estimations.

### Total sperm motility, viability, count and abnormality

Epididymis was minced in 1 ml phosphate buffer saline (pH 7.4) and centrifuged at 3000 rpm for 10 min to collect the seminal plasma and the spermatozoa. A simple grading system was used for assessment of sperm motility according to the WHO guidelines,<sup>[13]</sup> for which a drop of liquefied semen was placed on a glass slide, covered with a coverslip and observed under microscope at  $\times 400$ . The number of motile and non-motile spermatozoa was counted from different fields of slide. A total of 200 spermatozoa were counted and the mean of counts was calculated per sample.

Sperm viability (vitality) was estimated by assessing the membrane integrity of the cells. The percentage of live spermatozoa was assessed by identifying those with an intact cell membrane from dye exclusion.<sup>[14]</sup> One drop of semen was mixed with two drops of 1% eosin Y on a clean glass slide. After 30 s, three drops of 10% nigrosin solution were added to the above slide and mixed. A drop of the semen–eosin–nigrosin mixture was placed on a clear glass slide and a smear was prepared. It was allowed to air-dry. Then, unstained (live) and stained (dead) spermatozoa were counted under the microscope.

The number of spermatozoa was calculated from the concentration of spermatozoa.<sup>[13]</sup> The 100  $\mu\text{m}$  deep haemocytometer (Neubauer chamber) was used. 1:10 dilution of semen was made with the sperm diluents (sodium bicarbonate [ $\text{NaHCO}_3$ ] 50 g, formaldehyde solution [36%–40%] 10 ml [v/v], trypan blue 0.25 g and distilled water to make 1000 ml). Haemocytometer was charged with the sample. The number of sperms was counted on the central square of the chamber which has 25 large squares each further subdivided into 16 small subsquares.

For identifying and calculating sperm shape abnormalities, a fine epididymal sperm suspension was made and stained with 0.2 ml of 1% aqueous eosin. One drop of stained suspension was placed on the clean slide, dried and mounted in DPX. Slides were examined for sperm head and tail abnormalities. One thousand sperms per animal were being scored by the method of Bairy *et al.*<sup>[15]</sup> The sum of counts of different abnormalities was expressed as total abnormal sperm count/1000 spermatozoa.

### Malondialdehyde

Malondialdehyde (MDA) content in the testis and semen of control and fluoride-treated rats was determined by the method of Ohkawa *et al.*<sup>[16]</sup> MDA is the end product of lipid peroxidation of polyunsaturated fatty acids, and

it is estimated utilising its reaction with thiobarbituric acid (TBA). Tissues were quickly removed and washed in ice-cold 0.9% sodium chloride (NaCl), dried on filter paper, weighed and homogenised in ice-cold 1.15% potassium chloride (20% W/V) at 7500 g for 10 min. The supernatant was aliquoted and used for the assay of lipid peroxidation. The pellet was discarded and the resulting clean supernatant was used for the enzyme assay. The sample (0.2 ml) was added to a reaction mixture of 3.3 ml (0.2 ml of 8.0% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid, 0.8 ml of 0.8% butylated hydroxytoluene and 1.5 ml of 0.8% of TBA). Then sample was heated in a boiling water bath at 95°C for 60 min. After incubation, it was cooled to room temperature and centrifuged at 2000 rpm for 10 min and absorbance was measured at 553 nm. The concentration of MDA was expressed as nmol/mg protein.

### Superoxide dismutase

The activity of superoxide dismutase (SOD) in the testis and semen of control and fluoride-treated rats was determined by the method of Das *et al.*<sup>[17]</sup> Tissue was homogenised in 10 ml of 0.25 M sucrose solution and centrifuged at 2500 rpm for 10 min at 40°C–200°C. Three test tubes were marked as sample (A), blank (B) and control (C). In test tube 'A', 0.1 ml of sample and, in 'B' and 'C', 0.1 ml of distilled water were taken. After 5 min of incubation, 80 µl of riboflavin was added to sample and control test tubes. In the blank test tube, 80 µl of buffer was added after 5 min of incubation. All the samples were incubated for 10 min in SOD box and then 1 ml of Griess reagent was added to all the three tubes. SOD scavenges superoxide radicals that are produced by photoreduction of riboflavin. These superoxide radicals are then allowed to react with hydroxylamine hydrochloride to produce nitrite. The nitrite in turn reacts with sulphanic acid to produce a diazonium compound, which subsequently reacts with naphthylamine to produce a red azo Compound, absorbance of which was measured at 543 nm. The activity was expressed as unit/mg protein.

### Catalase

The activity of catalase (CAT) in the testis and semen of treated rats was determined by the method of Aebi.<sup>[18]</sup> Tissue was homogenised in 0.1 M phosphate buffer (pH 7.0) and centrifuged at 1500 rpm for 10 min. The supernatant was used for the assay. Two millilitres of buffer was added to a test tube and then added 50 µl of sample and 0.5 ml of H<sub>2</sub>O<sub>2</sub>. The decrease in absorbance was read at 0 time after 30 and 60 s at 240 nm using a UV-visible spectrophotometer. The enzyme activity was expressed as n moles H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein.

### Statistical analysis

The mean ± SE of each parameter was computed considering the data on six rats per group ( $n = 6$ ), and the data were analysed using complete randomised design SAS 9.4 and Statistical Package for the Social Sciences (SPSS), International Business Machines Corporation (IBM), Armonk, New York and judged significant if  $P < 0.05$ .

### RESULTS

There was a significant decrease in the body ( $F = 78.89$ ,  $P < 0.0001$ ) and organ (testis:  $F = 26.35$ ,  $P < 0.0001$ ; epididymis:  $F = 26.43$ ,  $P < 0.0001$ ) weight of rats after 40 days of fluoride administration, as compared to control, with maximum percentage (75%) decline in Group III [Table 1].

### Data report

The mean values (± standard deviation) of sperm quality and oxidative stress parameters in fluoride intoxicated rats are reported in Table 2. The data were statistically calculated by one-way analysis of variance followed by Bonferroni multiple comparison test.

### Correlation analysis

Data obtained were analysed by linear regression procedure, and the related correlation coefficients are depicted in Table 3. In testis, MDA was negatively correlated with sperm motility ( $r = -0.852$ ,  $P = 0.001$ ), viability ( $r = -0.879$ ,  $P = 0.001$ ) and count ( $r = -0.895$ ,  $P = 0.001$ ) but positively correlated with abnormalities ( $r = +0.962$ ,  $P = 0.001$ ), while SOD and CAT were positively correlated with sperm motility ( $r = +0.882$ ,  $P = 0.001$ ;  $r = +0.799$ ,  $P = 0.001$ ), viability ( $r = +0.835$ ,  $P = 0.001$ ;  $r = +0.829$ ,  $P = 0.001$ ) and count ( $r = +0.870$ ,  $P = 0.001$ ;  $r = +0.829$ ,  $P = 0.001$ ) but negatively correlated with abnormalities ( $r = -0.834$ ,  $P = 0.001$ ;  $-0.825$ ,  $P = 0.001$ ).

In semen also, MDA was negatively correlated with sperm motility ( $r = -0.864$ ,  $P = 0.001$ ), viability ( $r = -0.907$ ,  $P = 0.001$ ) and count ( $r = -0.903$ ,  $P = 0.001$ ) but positively correlated with abnormalities ( $r = +0.963$ ,  $P = 0.001$ ), while SOD and CAT were positively correlated with sperm motility ( $r = +0.855$ ,  $P = 0.001$ ;  $r = +0.833$ ,  $P = 0.001$ ), viability ( $r = +0.809$ ,  $P = 0.001$ ;  $r = +0.780$ ,  $P = 0.001$ ) and count ( $r = +0.872$ ,  $P = 0.001$ ;  $r = +0.840$ ,  $P = 0.001$ ) but negatively correlated with abnormalities ( $r = -0.863$ ,  $P = 0.001$ ;  $-0.812$ ,  $P = 0.001$ ).

### Sperm abnormalities

In control rats, spermatozoa with hooked head and straight tail were found [Figure 1].

In the NaF-treated group, abnormalities in spermatozoa included double and multiple tail and distorted neck,

**Table 1: Mean body and organ weight (g) of control and fluoride-treated rats**

	Treatment groups	Dose (ppm fluoride/kg bw)	Body/organ weight (g) (mean±SD)	Percentage decline	CRD analysis
Body weight	Control	1 ppm deionised water	166.67±13.66		$F=78.89$
	I	100	75.00±5.478***	55.00	$P<0.0001$
	II	200	61.67±7.528***	63.00	(CD=11.456)
	III	300	41.67±7.528***	75.00	
Testis	Control	1 ppm deionised water	1.097±0.1294		$F=26.35$
	I	100	0.682±0.0591***	37.83	$P<0.0001$
	II	200	0.537±0.0845***	51.05	(CD=0.1224)
	III	300	0.410±0.0632***	62.63	
Epididymis	Control	1 ppm deionised water	0.607±0.0761		$F=26.43$
	I	100	0.420±0.0303***	30.81	$P<0.0001$
	II	200	0.325±0.0187***	46.46	(CD=0.0568)
	III	300	0.243±0.0378***	59.97	

\*\*\* $P<0.0001$ . SD=Standard deviation, CRD: Complete randomised design, CD=Critical Difference

**Table 2: Mean±standard deviation of sperm motility, viability, count and abnormality, malondialdehyde, superoxide dismutase and catalase**

	Control	I	II	III
Sperm motility (%)	83.80±1.70	52.26±1.851***	45.18±2.066***	36.40±1.536***
Sperm viability (%)	89.17±4.71	56.83±7.96***	44.83±6.795***	30.67±5.279***
Sperm count (millions/ml)	17.44±0.84	9.3833±0.638***	6.11±0.548***	3.32±0.419***
Sperm abnormality (%)	18.00±1.79	34.67±3.933	58.00±3.033***	78.50±5.050***
Testis				
MDA	0.032±0.008	0.077±0.010***	0.149±0.019***	0.256±0.014***
SOD	2.074±0.573	1.261±0.203**	0.982±0.077***	0.657±0.047***
CAT	5.214±0.621	3.951±0.739*	3.052±0.608***	2.512±0.870***
Seminal plasma				
MDA	0.018±0.005	0.062±0.005***	0.102±0.009***	0.183±0.017***
SOD	2.027±0.544	1.474±0.158*	1.014±0.068***	0.783±0.046***
CAT	5.388±0.825	3.785±0.503**	3.077±0.722***	2.519±0.883***

\* $P<0.05$ , \*\* $P<0.001$ , \*\*\* $P<0.0001$ . MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase

coiled, folded, constricted and hairpin looped and head abnormalities such as double head, banana shape, small head, amorphous, bent head, curved hook and bend neck were prevalent [Figures 2-7].

## DISCUSSION

The present study investigated the relation between oxidative stress and sperm quality factors in rats treated with three different doses of fluoride. With an increase in the dose of fluoride, there was a decrease in both body and organ weight, which is in accordance with the previous study<sup>[19]</sup> in male albino rats, rabbits and mice fed on fluoride.<sup>[20,21]</sup> A decrease in body and testicular weight was also reported in albino rats intoxicated with groundwater fluoride.<sup>[22,23]</sup> The reported decrease in the reproductive organ weight could be due to Leydig cell atrophy resulting from oxidative damage induced in rat testis that led to a decrease in testosterone level.

Reproductive anomalies due to toxic exposure in males are one of the fastest growing areas of concern in toxicology. Fluoride ion is able to exert powerful



**Figure 1:** Photomicrograph of cauda epididymal spermatozoa of control rat. Eosin ×1000 (under oil immersion lenses)

effects on various enzymes that affect the status of oxidant/antioxidant systems in living organisms. The present study demonstrated that fluoride exposure leads to oxidative stress as indicated by an increased level of

**Table 3: Correlation coefficients (*r*) of malondialdehyde, superoxide dismutase and catalase with sperm quality parameters**

	Testis			Semen		
	MDA	SOD	CAT	MDA	SOD	CAT
Sperm motility	-0.852***	+0.882***	+0.799***	-0.864***	+0.855***	+0.833***
Sperm viability	-0.879***	+0.835***	+0.829***	-0.907***	+0.809***	+0.780***
Sperm count	-0.895***	+0.870***	+0.829***	-0.903***	+0.872***	+0.840***
Sperm abnormality	+0.962***	-0.834***	-0.825***	+0.963***	-0.863***	-0.812***

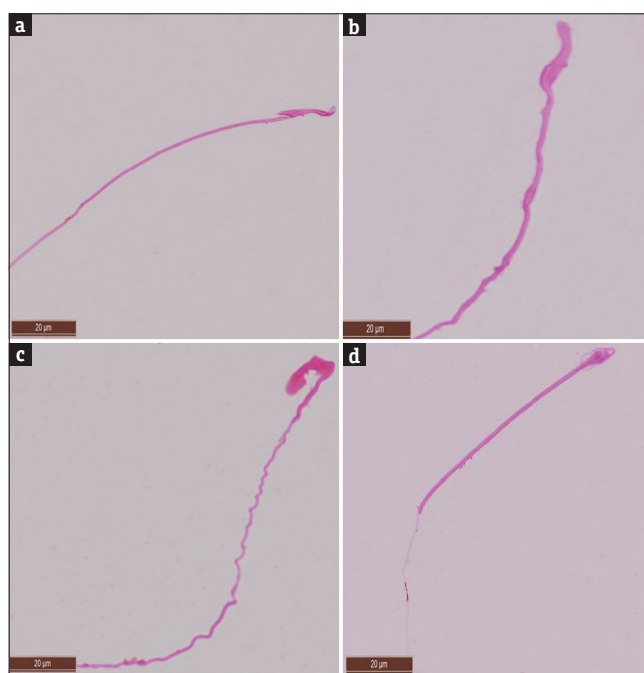
\*\*\* $P \leq 0.001$  level (two-tailed). MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase



**Figure 2:** Photomicrographs of cauda epididymal spermatozoa with coiled tail (a and c) and with hairpin loop (b and d). Eosin  $\times 1000$  (under oil immersion lenses)

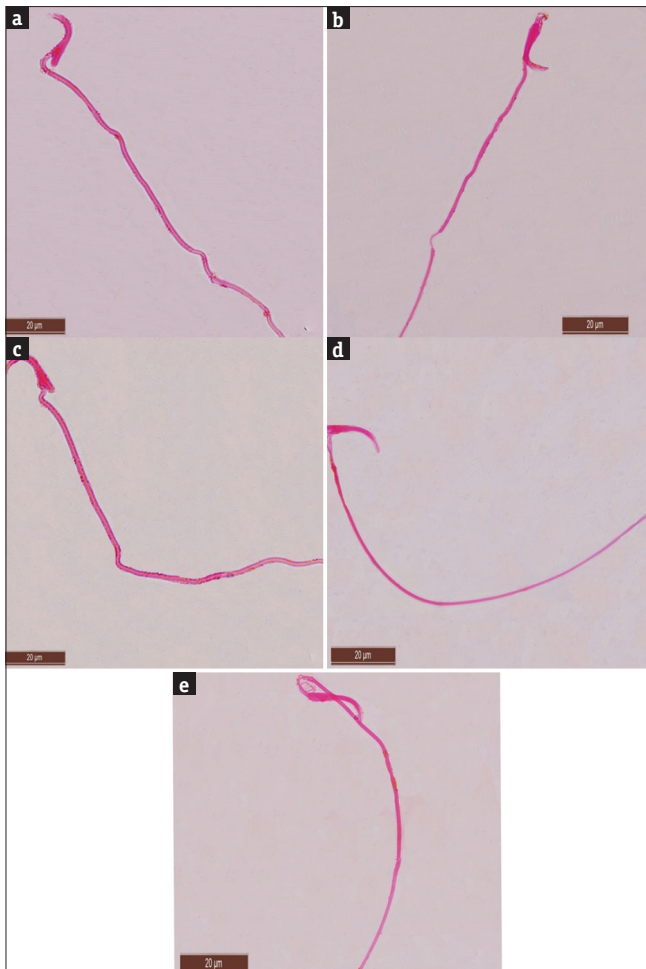
MDA in the testis and seminal plasma with respect to control.

The present study revealed that exposure of rats to sodium fluoride significantly elicited the elevation of MDA content in testis and seminal plasma indicating the close relationship between fluoride-induced male testicular toxicity and oxidative stress. MDA is an important reactive metabolite and an indicator of lipid peroxidation which disturbs the integrity of cellular membranes leading to the leakage of cytoplasmic enzymes.<sup>[24]</sup> Lipid peroxidation has been suggested to be important in contributing to mitochondrial dysfunction rather than the inhibition of mitochondrial electron transport.<sup>[25]</sup> Moreover, oxidative stress generated by free radicals and hydrogen peroxide is greater if fluoride hinders the production of the free radical indices of the defence system.<sup>[26]</sup> Reduction in these indices has been found demonstrated in animals subjected to fluoride toxicity as well as in people living in endemic areas.<sup>[27,28]</sup> Generation of free radicals and reactive oxygen species (ROS) is a continuous process in the body, and to counteract their devastating effects,



**Figure 3:** Photomicrographs of cauda epididymal spermatozoa of rats with curved hook (a), without hook (b), head bent and constricted tail (c) and with amorphous head and short tail (d). Eosin  $\times 1000$  (under oil immersion lenses)

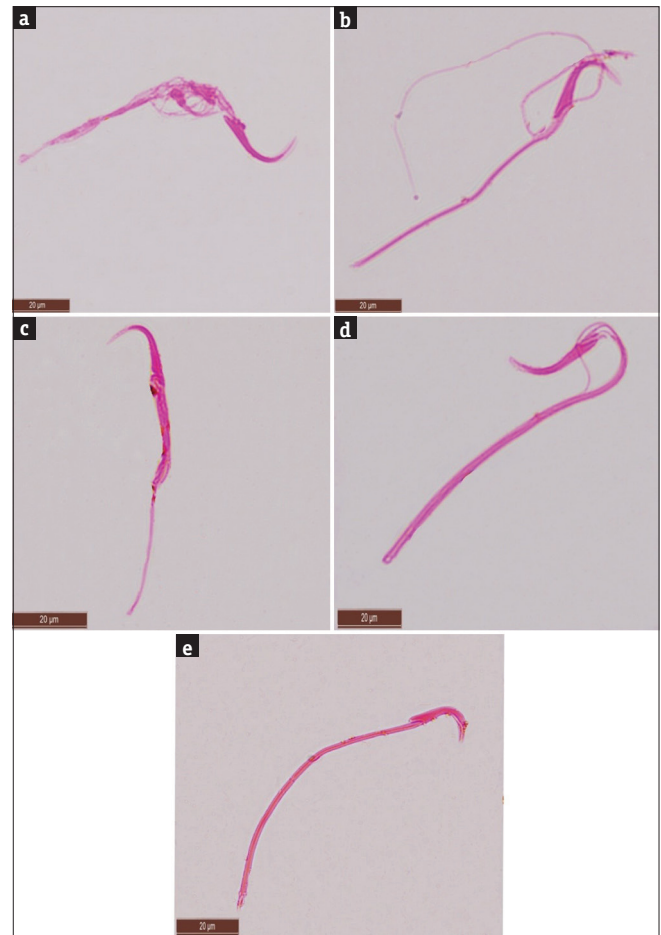
mammalian cells are bestowed with considerable antioxidant defence mechanisms consisting of enzymatic action by SOD, CAT, glutathione peroxidase (GPx) and glutathione-S-transferase (GST).<sup>[29,30]</sup> In the present study, it was observed that sodium fluoride exposure in rats caused a significant ( $P < 0.0001$ ) decrease in activity of SOD and CAT in testis and seminal plasma. Further correlation analyses have revealed a negative correlation existed between the level of fluoride and the activity of antioxidant enzymes. As the level of fluoride increases in testis and seminal plasma, the activity of SOD and CAT decreases. The present study demonstrates marked changes in all oxidative stress markers in test rats which may be due to the overproduction of ROS in male reproductive tract and may be the potential cause of sperm dysfunctioning. The significant reduction in antioxidant enzymes is in accordance with previous studies.<sup>[31]</sup> In consonance with our study, increased lipid peroxidation along



**Figure 4:** Photomicrographs of cauda epididymal spermatozoa of rats with bent neck (a and c), bent head (b) and with distorted neck (d and e). Eosin  $\times$  1000 (under oil immersion lenses)

with decrements in antioxidant parameters such as GPx, glutathione, total ascorbic acid, GST, glutathione reductase, SOD and CAT levels were found to affect testis function in fluoride treated rats.<sup>[30]</sup>

SOD is a part of the first line of defence mechanism and catalyses the reaction between superoxide anion and hydrogen peroxide to form molecular oxygen and water. Decreased activity of SOD in the present study is suggestive of its excessive utilisation for neutralising superoxide generated by fluoride. The CAT enzyme is important in the removal of hydrogen peroxide generated by SOD. In addition, CAT activity is inhibited by superoxide radicals.<sup>[32]</sup> Decreased CAT activity in the present study is indicative of excess production of H<sub>2</sub>O<sub>2</sub> and other hydroperoxide radicals, owing to its immoderate utilisation. This is in accordance with a previous study where the effect of oxidative stress on the apoptosis of Sertoli cells induced by NaF and the reported enhanced ROS levels were observed with an increase in NaF dose.<sup>[33]</sup> In



**Figure 5:** Photomicrographs of cauda epididymal spermatozoa with deformed tail region (a-e). Eosin  $\times$  1000 (under oil immersion lenses)

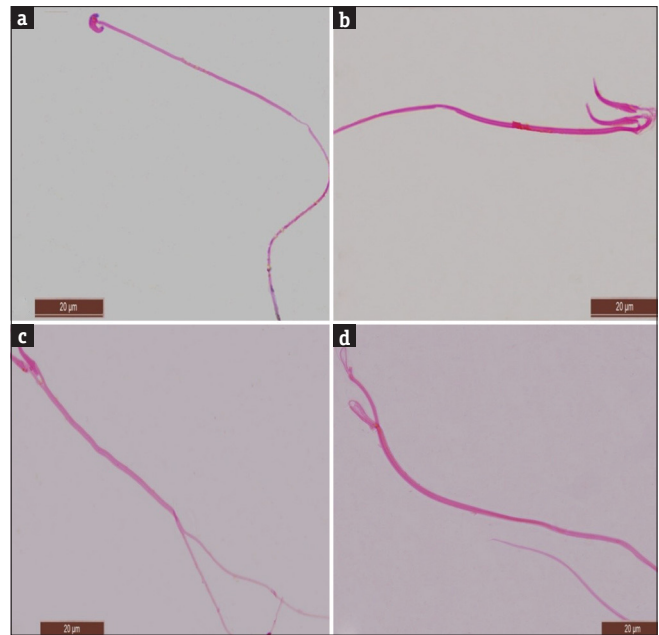
addition, the content of MDA significantly increased in the 12 and 24  $\mu$ g/ml NaF groups, whereas there was a marked decrease in the SOD activity of the NaF-treated groups.

In the present study, a negative correlation was observed between the level of MDA and sperm motility, viability and count, while a positive correlation was observed between the level of MDA and sperm abnormalities. On the other hand, there was a positive correlation between the activities of both SOD and CAT and sperm motility, viability and count, while there was a negative correlation between the activities of both SOD and CAT and sperm abnormalities. Sperm plasma membrane is composed of polyunsaturated fatty acids, and they are highly susceptible to lipid peroxidation and protein oxidation.<sup>[34]</sup> Fluoride decreases in the activities of SOD and CAT.<sup>[35]</sup> Studies have also revealed that SOD and CAT in testis and sperm protect sperm from oxidative attack and the deficiency of which correlated with male infertility and sperm quality.<sup>[36]</sup>



**Figure 6:** Photomicrographs of cauda epididymal spermatozoa with deformed tail region (a-e). Eosin  $\times 1000$  (under oil immersion lenses)

The effect of fluoride on SOD activity in spermatozoa may be by acting as an inhibitor of Mn-SOD and Cu/Zn SOD, which involves its binding to the divalent cofactors in active site on SOD. SOD inactivation would lead to increased levels of superoxide anion within the mitochondria which could lead to oxidation of key mitochondrial proteins and ultimately mitochondrial dysfunction and cell death. The defective sperm with abnormalities in the head, midpiece and tail have a high ROS production.<sup>[36]</sup> Fluoride has been demonstrated to cause a significant increase in sperm abnormality, which provides a strong pathological basis for excessive ROS presence. ROS can directly attack unsaturated fatty acid on the sperm membrane, inducing lipid peroxidation, damaging the membrane integrity, destroying the structure of axoneme and finally reducing sperm activity and fertility.<sup>[11,37]</sup> Sperm with any abnormal morphology could result in low function, adversely impacting on the successful fertilisation. It was found that there was a significant ( $P < 0.001$ ) increase in the number of abnormal sperms in fluoride-intoxicated rats. The abnormalities observed were in head and



**Figure 7:** Photomicrographs of cauda epididymal spermatozoa small head (a), double head (b), double head and double tail (c) and amorphous double head (d). Eosin  $\times 1000$  (under oil immersion lenses)

tail region of sperm. Head abnormalities noted were bent head, double, small, amorphous, banana shaped, without hook and tail. Tail abnormalities included short tail, curved, hairpin looped, coiled, multiple and tails without heads. In the present investigation, the sperm count, motility and viability of fluoridated groups were reduced significantly ( $P < 0.0001$ ), which is in accordance with the previous studies,<sup>[38]</sup> where exposure to high concentrations of NaF in drinking water led to a decreased sperm count, sperm motility and sperm survival and an increased sperm abnormality in male mice.<sup>[3,39]</sup> Fluoride decreases sperm motility by either direct effect on the motile apparatus without affecting other metabolic systems or could be due to decline in the fructose level, which provides energy for motility, due to alteration in carbohydrates metabolism. Fluoride also binds with cofactors such as Mg, Ca, Zn and Se and thus inhibits glycolysis, respiration and motility of sperms.<sup>[40]</sup>

## CONCLUSION

The present experimental study indicates that fluoride toxicity produces definite effects on spermatozoa which are evident from morphological abnormalities and alterations in sperm quality parameters. These morphological anomalies may be induced by the mechanism of oxidative stress and interference in antioxidant defence mechanism.

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## Financial support and sponsorship

This study was financially supported by DST-INSPIRE.

## Conflicts of interest

There are no conflicts of interest.

## Data availability statement

The data sets in this study are available with the author upon reasonable request.

## REFERENCES

- Agency for Toxic Substances and Disease Registry. Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine (update). Georgia, USA: Agency for Toxic Substances and Disease Registry; 2003.
- Pervez A, Sinha MK, Yadav M. Implications of fluoride toxicity on the male reproductive system: A review. *J Mt Res* 2016;11:1-7.
- Wan S, Zhang J, Wang J, Shanxi B. Effects of high fluoride on sperm quality and testicular histology in male rats. *Fluoride* 2006;39:17-21.
- Losano J, Angrimani D, Dalmazzo A, Rui BR, Brito MM, Mendes CM, *et al.* Effect of mitochondrial uncoupling and glycolysis inhibition on ram sperm functionality. *Reprod Domest Anim* 2017;52:289-97.
- Henkel RR. Leukocytes and oxidative stress: Dilemma for sperm function and male fertility. *Asian J Androl* 2011;13:43-52.
- Kasai T, Ogawa K, Mizuno K, Nagai S, Uchida Y, Ohta S, *et al.* Relationship between sperm mitochondrial membrane potential, sperm motility, and fertility potential. *Asian J Androl* 2002;4:97-103.
- Rezaee-Tazangi F, Zeidooni L, Rafiee Z, Fakhredini F, Kalantari H, Alidadi H, *et al.* Taurine effects on Bisphenol A-induced oxidative stress in the mouse testicular mitochondria and sperm motility. *JBRA Assist Reprod* 2020;24:428-35.
- Wang X, Sharma RK, Gupta A, George V, Thomas AJ, Falcone T, *et al.* Alterations in mitochondria membrane potential and oxidative stress in infertile men: A prospective observational study. *Fertil Steril* 2003;80 Suppl 2:844-50.
- Agnihotri SK, Agrawal AK, Hakim BA, Vishwakarma AL, Narender T, Sachan R, *et al.* Mitochondrial membrane potential (MMP) regulates sperm motility. *In Vitro Cell Dev Biol Anim* 2016;52:953-60.
- Barbagallo F, La Vignera S, Cannarella R, Aversa A, Calogero AE, Condorelli RA. Evaluation of sperm mitochondrial function: A key organelle for sperm motility. *J Clin Med* 2020;9:363.
- Kao SH, Chao HT, Chen HW, Hwang TI, Liao TL, Wei YH. Increase of oxidative stress in human sperm with lower motility. *Fertil Steril* 2008;89:1183-90.
- Nowicka-Bauer K, Nixon B. Molecular changes induced by oxidative stress that impair human sperm motility. *Antioxidants (Basel)* 2020;9:134.
- World health organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. Geneva: V edn; 2010. p. 21-101.
- Eliasson R. Supravital staining of human spermatozoa. *Fertil Steril* 1977;28:1257.
- Bairy L, Paul V, Rao Y. Reproductive toxicity of sodium valproate in male rats. *Indian J Pharmacol* 2010;42:90-4.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
- Das K, Samanta L, Chainy GB. A modified spectrophotometric assay for superoxide dismutase using nitrite formation by superoxide radicals. *Indian J Biochem Biophys* 2000;37:201-4.
- Aebi HE. Catalase. In: Bergmeyer HU, editor. *Methods of Enzymatic Analysis*. Vol. 3. Weinhei: Verlag Chemie; 1983. p. 273-86.
- Das SK, Karmakar SN, Roy DS. Efficacy of ascorbic acid as protective substance on sodium fluoride induced reproductive impairment. *European J Biomed Pharma Sci* 2016;3:265-71.
- Kumar N, Sood S, Arora B, Singh M, Beena. Effect of duration of fluoride exposure on the reproductive system in male rabbits. *J Hum Reprod Sci* 2010;3:148-52.
- Singh PK, Feroz AD, Sheeba H, Khalil A, Samir AM. Effect of tamarindus indica on the testes of albino rat after fluoride intoxication. *Int J Pharma Bio Sci* 2012;3:487-93.
- El-Metwally AE, El-Hallawany HA, Ali AH. Immunohistopathologic study on the ameliorative effect of propolis against fluoride cytotoxicity on rabbit buck fertility. *Alex J Vet Sci* 2017;53:156-66.
- Feng D, Huang H, Yang Y, Yan T, Jin Y, Cheng X, *et al.* Ameliorative effects of N-acetylcysteine on fluoride-induced oxidative stress and DNA damage in male Rats' testis. *Mutat Res Genet Toxicol Environ Mutagen* 2015;792:35-45.
- McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocyte (hemocuprein). *J Biol Chem* 1969;244:6049-55.
- Sen T, Sen N, Tripathi G, Chatterjee U, Chakrabarti S. Lipid peroxidation associated cardiolipin loss and membrane depolarization in rat brain mitochondria. *Neurochem Int* 2006;49:20-7.
- Rzeuski R, Chlubek D, Machoy Z. Interactions between fluoride and biological free radical reactions. *Fluoride* 1998;31:43-5.
- 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;230:352-7.
- Baba NA, Raina R, Verma PK, Sultana M, Prawez S, Nisar NA, *et al.* Toxic effects of fluoride and chlorpyrifos on antioxidant parameters in rats: Protective effects of vitamin C and E. *Fluoride* 2013;46:73-9.
- Dubey N, Raina R, Khan AM. Toxic effects of deltamethrin and fluoride on antioxidant parameters in rats. *Fluoride* 2012;45:242-6.
- Rao MV, Bhatt RN. Protective effect of melatonin on fluoride-induced oxidative stress and testicular dysfunction in rats. *Fluoride* 2012;45:116-24.
- Zini A, Garrels K, Phang D. Antioxidant activity in the semen of fertile and infertile men. *Urology* 2000;55:922-6.
- Kono Y, Fridovich I. Superoxide radical inhibits catalase. *J Biol Chem* 1982;257:5751-4.
- Yang Y, Lin X, Huang H, Feng D, Ba Y, Cheng X, *et al.* Sodium fluoride induces apoptosis through reactive oxygen species-mediated endoplasmic reticulum stress pathway in Sertoli cells. *J Environ Sci (China)* 2015;30:81-9.
- Chauhan DS, Singh VP, Tripathi S, Tomar A, Tiwari M, Tomar S, *et al.* Fluoride reduces semen quality at risk level in high fluoride affected regions. *Int J Biol Med Res* 2013a; 4:3410-3.
- Chauhan DS, Tomar A, Tiwari M, Singh VP, Tomar S, Tripathi S. Endogenous and exogenous antioxidants status in seminal plasma of skeletal fluorotic patients. *Sch J App Med Sci* 2013b; 1:152-7.



36. Agarwal A, Said TM. Oxidative stress, DNA damage and apoptosis in male infertility: A clinical approach. *BJU Int* 2005;95:503-7.
37. Saleh RA, Agarwal A. Oxidative stress and male infertility: From research bench to clinical practice. *J Androl* 2002;23:737-52.
38. Huang C, Niu R, Wang J. Toxic effects of sodium fluoride on reproductive function in male mice. *Fluoride* 2007;40:162-8.
39. Cui LX, Jiang CX, Wang XL, Chen XM. Experimental study on effect of fluoride on reproductive system of male rats. *Chin J Endemiol* 2003;22:195-7.
40. Huang C, Yang H, Niu R, Sun Z, Wang J. Effect of sodium fluoride on androgen receptor expression in male mice. *Fluoride* 2008;41:10-7.