

EFFECTS OF VITAMIN C AND CALCIUM ON THE REVERSIBILITY OF FLUORIDE-INDUCED ALTERATIONS IN SPERMATOZOA OF RABBITS

by

N.J. Chinoy*, E. Sequeira and M.V. Narayana
Ahmedabad, India

SUMMARY: The present study was designed to investigate the effects of fluoride on the metabolism and functions of cauda epididymal spermatozoa of rabbits. The studies on reversibility of fluoride-induced effects by fluoride withdrawal, Vitamin C (ascorbic acid, AA) or Calcium (Ca^{+2}) and combined AA + Ca^{+2} ingestion were also investigated.

Adult Male rabbits, Group II and III, were fed 20 and 40 mg/kg body weight sodium fluoride, respectively, for 30 days. Afterwards, cauda epididymal spermatozoa were obtained by micropuncture technique. Alterations in the activities of some specific androgen-dependent enzymes on sperm namely, ATPase, ACP, SDH, and protein, as well as reduction in Na^{+} and K^{+} levels in the spermatozoa, might be due to altered and hostile internal milieu of the epididymis in NaF-treated rabbits. Reduction in sperm motility, count, and changes in their morphology and metabolism led to the significant decline in fertility of the treated animals. After withdrawal of NaF treatment for 30 days (Group IV), no recovery was obtained in all the parameters which were altered, including fertility rates.

During the withdrawal period, AA or Ca^{+2} and combined AA + Ca^{+2} were administered to Groups V, VI, and VII, respectively. With both AA or Ca^{+2} marked recovery occurred from all induced effects. The extent of recovery, however, was somewhat more pronounced by AA treatment than that brought about by Ca^{+2} administration during the withdrawal period. The combined treatment with AA + Ca^{+2} manifested a synergistic effect for recovery of all parameters. The effects of fluoride are therefore transient and reversible, in agreement with earlier data. Moreover, AA and Ca^{+2} have therapeutic importance in fluorotic animals. These findings have a direct bearing on human subjects exposed to high fluoride levels.

KEY WORDS: Ascorbic acid; Calcium; Fluoride; Spermatozoa; Vitamin C.

Introduction

The clinical manifestations of fluorosis due to excessive ingestion of fluoride are fairly well documented. Some studies have reported the effects of fluoride on soft tissue (1), but there is a paucity of data on reproductive organs, a topic which is rather controversial (2,3). Chinoy and Sequeira (4-6)

* Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad 380009, India.

have reported that reproductive organs of mice were affected by sodium fluoride (NaF) ingestion (10 and 20 mg/kg body weight) for 30 days. A decrease in sperm density and sperm motility which occurred, led to loss of fertility. The sperm acrosomal integrity, morphology, and metabolism were altered in NaF-treated mice. However, fluoride-induced changes were transient and reversible after 2 months of withdrawal of treatment. A microdose of NaF when directly injected, in retrograde direction, into the distal vas deferens of rats also caused alterations in structure of reproductive organs and their metabolism as well as reduction in fertility (7). The present study was designed to investigate the effects of fluoride on the metabolism of cauda epididymal spermatozoa of rabbits in light of earlier data. The studies on the reversibility of induced effects by NaF withdrawal, vitamin C or calcium ingestion and combined vitamin C + calcium administration during the withdrawal period were also investigated.

Materials and Methods

Adult rabbits (*Oryctolagus cuniculus*) weighing between 1 and 1.8 kg were divided into seven groups (Table 1). The first group was given the control diet. The next three groups of animals were fed sodium fluoride (NaF) orally at doses of 20 and 40 mg/kg body weight/day respectively for 30 days. At the end of the treatment period, epididymal fluid was collected by micro-puncture technique. It was used in studying the microenvironment of epididymis (8). After appropriate dilution of the luminal contents, the sample was centrifuged to separate luminal fluid and sperm suspension; they were utilized for requisite biochemical analysis. The same animals were utilized for withdrawal

Table 1
Experimental Protocol

Group	Treatment	Duration (days)	Day of Necropsy*	No. of Animals
I	Control	30	Along with treated	5
II	NaF 20 mg/kg B.W./day/animal	30	31	5
III	NaF 40 mg/kg B.W./day/animal	30	31	5
IV	NaF 40 mg/kg B.W./day/animal then withdrawal for 1 month	30	31	5
V	NaF then withdrawal 1 month then vitamin C 100 mg/kg B.W./day/animal	30	31	5
VI	NaF then withdrawal 1 month + Ca 125 mg/day/animal	30	31	5
V	NaF then withdrawal 1 month + Vitamin C 100 mg/kg B.W. + Ca 125 mg/day/animal	30	31	5

* From beginning of withdrawal period.

studies. NaF treatment was discontinued for 30 days to Group IV; to Group V rabbits ascorbic acid (AA) at the dose of 100 mg/kg body weight and to another group (Group VI) osteocalcium tablets containing 125 mg calcium were administered. To Group VII, combined AA + Ca⁺⁺ was administered for 30 days during the withdrawal period. At the end of 30 days the cauda epididymal fluid was collected and subjected to further procedure as in the case of control and NaF-treated animals.

The percent sperm motility and sperm count of the epididymis from control and all treated and withdrawal groups of rabbits were determined by means of a Neubauer chamber of the Haemocytometer according to the method of Prasad *et al.* (9) and expressed as percentages and millions/mL, respectively. The acrosomal integrity of the sperm from the cauda epididymis of control, treated, and withdrawal groups was studied using the modified silver nitrate technique (10).

The following parameters were investigated in sperm suspension of all the experimental groups:

- The enzyme adenosine triphosphatase (ATPase) activity was assayed following the method of Quinn and White (11). The enzyme ATPase hydrolyses the substrate ATP into adenosine diphosphate and inorganic phosphate (ip). The ip formed at the end of the incubation was assayed to determine the rate of the reaction.
- The activity of succinate dehydrogenase (SDH) was determined by the method of Beatty *et al.* (12) and expressed as μg formazan/mL/30 minutes.
- The acid phosphatase (ACP) activity was assayed by the method of Bessey *et al.* (13) and was expressed as μ moles of p-nitrophenol liberated/mL/30 minutes.
- The protein content was determined by the method of Lowry *et al.* (14) and expressed as μg of protein/mL of sperm suspension.
- The sodium and potassium contents in the epididymal spermatozoa were estimated by the Systronics Flame Photometer, Digital Unit type 125, according to the method of Dean (15).

Results

Body Weight: Body weight decreased after NaF treatment in comparison to the controls. Recovery occurred throughout the withdrawal period in rabbits of Groups V to VII only. No recovery occurred in Group IV animals from which NaF treatment was withdrawn for one month. However, recovery was significantly greater with vitamin C (AA) supplementation than with calcium. Body weight recovered significantly with the administration of vitamin C and calcium together (Group VII) during the recovery period (Table 2).

Sperm Motility: The percent of cauda epididymal sperm motility decreased significantly ($p < 0.001$) in a dose related pattern, after NaF treatment compared to control (Table 2). Sperms were sluggishly motile. Head to head agglutination and deflagellation was also observed.

Sperm motility was recovered by vitamin C as well as by calcium ingestion. However, recovery was more significant by vitamin C than by calcium. Moreover, the synergistic effect of vitamin C and calcium was manifested to enhance sperm motility during the recovery period (Group VII) (Table 2). No recovery was observed in Group IV, i.e. withdrawal of NaF.

Sperm Count: The cauda epididymal sperm count of treated rabbits decreased with both doses of NaF compared to the controls. The sperm count recovered more by vitamin C in comparison to calcium treatment (Table 2). The sperm count further recovered almost to control level by the combined treatment with vitamin C and calcium (Table 2), but no recovery occurred by withdrawal of treatment alone.

Fertility Rate: The fertility rate was 95% positive in control rabbits. However, after administration of NaF (both doses), it was reduced to 33% and 0% respectively ($p < 0.001$) compared to the controls. Combined treatment with vitamin C and calcium during the withdrawal period resulted in significant recovery of fertility compared with the individual treatments of vitamin C or calcium but not by withdrawal alone (Table 2).

Adenosine Triphosphatase (ATPase): Significantly decreased ($p < 0.001$) spermatozoal ATPase activity was observed due to the fluoride treatment. However, administration of vitamin C and calcium in combination, caused significant recovery. Moreover, vitamin C alone had more effect than that of calcium in recovery of the enzymic activity, which failed to recover by NaF withdrawal alone (Table 3).

Table 2

Showing Body Weight, Sperm Motility, Sperm Count and Fertility Rate of Control, NaF Treated, NaF Withdrawal, and Vitamin C, Calcium, and Vitamin C + Calcium to NaF Withdrawal Groups of Rabbits.

Group	Treatment	Body wt. (kg)	Sperm Motility (%)	Sperm Count ($10^6/mL$)	Fertility Rate (%)
I	Control	1.7 \pm 0.1	77 \pm 1.6	49 \pm 0.2	95-100
II	NaF 20 mg/kg B.W.	1.3 \pm 0.3	37 \pm 1.8*	36 \pm 1.2*	33*
III	NaF 40 mg/kg B.W.	1.1 \pm 0.3	27 \pm 2.0*	34 \pm 1.3*	0*
IV	NaF 40 mg/kg B.W. withdrawal 1 mo.	1.2 \pm 0.5	33 \pm 1.5	37 \pm 0.7	0*
V	NaF, Withdrawal, + Vitamin C	1.4 \pm 0.1	66 \pm 0.6	46 \pm 0.9	75*
VI	NaF, Withdrawal, Ca	1.3 \pm 0.1	49 \pm 1.3	40 \pm 0.2	67*
VII	NaF, Withdrawal + Vitamin C + Ca	1.8 \pm 0.1	72 \pm 1.1	48 \pm 0.9*	95-100

Values are Mean \pm S.E.

* $p < 0.001$

Table 3

Showing Adenosine Triphosphate (ATPase), Succinate Dehydrogenase (SDH), Acid Phosphate (ACP) and Protein Concentration in Spermatozoa of Control, NaF Treated, and NaF Withdrawal and of Vitamin C, Calcium and Vitamin C + Calcium to NaF Withdrawal Groups of Rabbits.

Group	Treatment	ATPase (μ mol ip/ mL/30')	SDH (μ g formazan/ mL/30')	ACP (μ mol p- nitrophenol/ mL/30')	Protein (μ g/mL)
I	Control	5.3 \pm 0.2	208 \pm 6.3	0.7 \pm 0.04	484 \pm 13
II	NaF 20 mg/kg B.W.	3.0 \pm 0.1*	112 \pm 3.7*	0.3 \pm 0.03*	303 \pm 16*
III	NaF 40 mg/kg B.W.	2.1 \pm 0.2*	78 \pm 1.3*	0.4 \pm 0.05*	286 \pm 7*
IV	NaF 40 mg, withdrawal 1 mo.	2.7 \pm 0.1	108 \pm 3.1	0.3 \pm 0.01	318 \pm 21
V	NaF, Withdrawal + Vitamin C	4.9 \pm 0.3	187 \pm 7.7	0.6 \pm 0.004	417 \pm 18
VI	NaF, Withdrawal + Calcium	3.1 \pm 0.2	131 \pm 10	0.4 \pm 0.02	386 \pm 17
VI	NaF, Withdrawal + Vitamin C + Ca	5.2 \pm 0.2*	201 \pm 3.1*	0.6 \pm 0.06*	477 \pm 10*

Values are Mean \pm S.E.

* $p < 0.001$

Succinate Dehydrogenase (SDH): Succinate dehydrogenase activity in the spermatozoa decreased significantly in treated rabbits compared to controls ($p < 0.001$). The decrease was dose dependent. During the recovery period, SDH activity recovered more by vitamin C than by calcium. Further, the synergistic therapeutic effect of vitamin C and calcium was greater in combination than the individual treatments or by NaF withdrawal alone (Table 3).

Acid Phosphatase (ACP): NaF treatment with both doses caused a significant decrease in acid phosphatase activity in spermatozoa. Administration of combined treatment of vitamin C and calcium resulted in recovery which was more significant than the individual treatments of vitamin C or calcium (Table 3). NaF withdrawal alone did not cause recovery of enzymic activity.

Protein: The protein concentration of spermatozoa also showed a significant dose-dependent decline ($p < 0.001$) after fluoride treatment. During the recovery period the protein concentration was enhanced by the combined administration of vitamin C and calcium. Moreover, the effect of vitamin C was more pronounced than that of calcium alone. However, NaF withdrawal (1 month) was not conducive to recovery (Table 3).

Na⁺ and K⁺ Levels: Na⁺ levels in the spermatozoa were significantly reduced throughout the treatment compared to the controls ($p < 0.001$) (Table 4). K⁺ levels in spermatozoa were also decreased significantly ($p < 0.001$) after

Table 4

Showing Na⁺ and K⁺ Levels in Spermatozoa of Control, NaF Treated, NaF Withdrawal, Vitamin C, Calcium and Vitamin C + Calcium to NaF Withdrawal Groups of Rabbits

Group	Treatment	Na ⁺ (ppm)	K ⁺ (ppm)
I	Control	144 ±5.3	162 ±4
II	NaF 20 mg/kg B.W.	123 ±2.6*	141 ±2
III	NaF 40 mg/kg B.W.	97 ±4.5*	96 ±5
IV	NaF 40 mg/kg B.W. withdrawal 1 mo.	100 ±3.5	116 ±5
V	NaF, Withdrawal + Vitamin C	121 ±3.3	129 ±3
VI	NaF, Withdrawal + Ca	102 ±3.9	147 ±3
VII	NaF, Withdrawal, + Vitamin C + Ca	139 ±6.3*	152 ±3*

Values are Mean ±S.E.

* p < 0.001

NaF treatment. During the withdrawal period both the treatments with vitamin C as well as with calcium administered individually had a significant effect on the recovery of Na⁺ and K⁺ levels of the spermatozoa. However, calcium produced a better recovery in the K⁺ levels than did vitamin C (Table 4). The combined treatment by vitamin C and calcium manifested a synergistic effect for recovery of both Na⁺ and K⁺ in sperm (Table 4). On the other hand, NaF withdrawal alone did not cause recovery.

Morphology of Spermatozoa - Silver-Nitrate Staining Technique: The silver-nitrate staining of cauda epididymal spermatozoa of rabbit revealed a clear differentiation of the acrosomal, post acrosomal and mid-piece regions (Figure 1). But in rabbits administered NaF, and staining was diffused with no proper demarcations. Deflagellation and agglutination of spermatozoa was also observed due to the effect of NaF (Figures 2 and 3). In withdrawal Group V and VI silver-nitrate staining

Figure 1

Cauda Epididymal Spermatozoa of Normal Rabbit Stained with Silver Nitrate Showing Clear Differentiation of Acrosomal and Post Acrosomal Regions. Note Intact Acrosomal Cap. x1735.

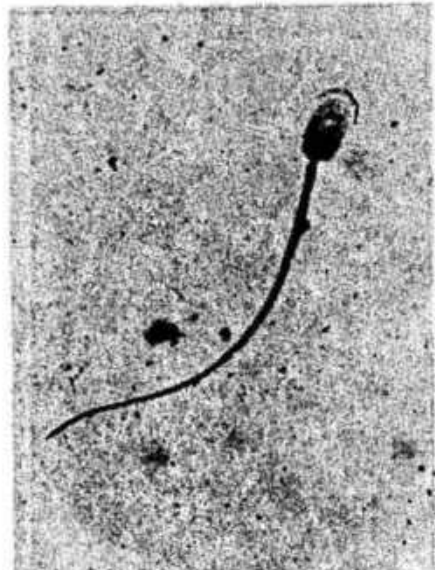


Figure 2

Cauda Epididymal Spermatozoa of Rabbit Treated with NaF 20 mg/kg Body Weight. Note Poor Differentiation of Acrosomal Region, Deflagellated Spermatozoa and Coiling of the Tail. x1450.

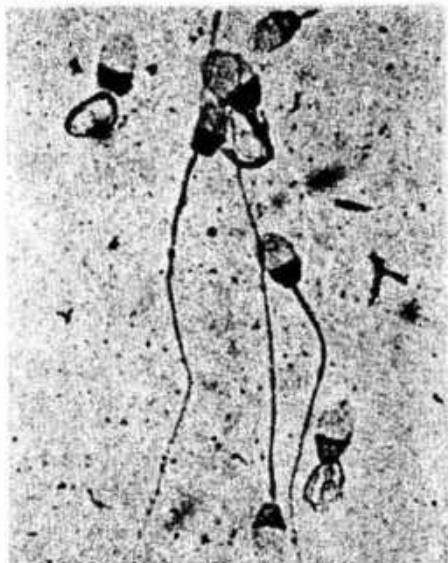


Figure 3

Silver-Nitrate Staining Showing Agglutination of Spermatozoa of Cauda Epididymis of Rabbit Treated with NaF 40 mg/kg Body Weight. x1450.



revealed spermatozoa which appeared quite normal (Figures 4 and 5).

Discussion

The present study was undertaken to investigate the effects of sodium fluoride (NaF) at a low and high dose (20 and 40 mg/kg body weight) on cauda epididymal spermatozoa of rabbits as well as to compare the extent of recovery after NaF withdrawal and the effects of vitamin C (AA) or calcium (Ca^{+2}) ingestion during the withdrawal period. The data revealed that NaF treatment resulted in a decrease in body weight as reported by others (16).

Fluoride ion is an inhibitor that has been extensively used in sperm metabolic studies. Schoff and Lardy (17) have demonstrated the effect of fluoride and caffeine on the metabolism and motility of ejaculated bull sperm. The sperm motility, glycolysis, and respiration could be altered by fluoride. Sperm treated with 30 mM fluoride became immobile within 2 minutes and the flagella assumed a linear, rod-like conformation. In the present study too, the motility of cauda epididymal spermatozoa decreased significantly in NaF-treated rabbits. Cauda epididymal sperm-ATPase activity was significantly affected throughout the NaF treatment. It has been reported that fluoride

Figure 4

Rabbit Cauda Epididymal Spermatozoa Treated with Vitamin C during Withdrawal Period. Clear Differentiation of Acrosomal and Post-Acrosomal Regions Are Observed Similar to Normal. x1650.

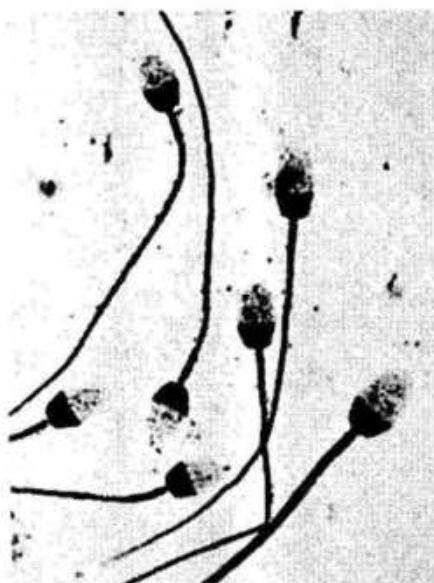


Figure 5

Cauda Epididymal Spermatozoa of Rabbit Treated with Calcium during Withdrawal Period Showing Recovery. x1650.



could directly inhibit the dynein ATPase in cilia (18), or else its decrease might be due to some structural alterations in the mitochondria in the mid-piece region, thereby causing a decrease in sperm motility. Therefore, in the future, ultrastructural studies of the sperm are necessary in NaF-treated animals.

The activity of succinate dehydrogenase (SDH), a mitochondrial oxidative enzyme also decreased in cauda epididymal spermatozoa after NaF treatment. It is likely that the mitochondrial structure and/or metabolism may have been affected, as mentioned above. Similar results were also obtained in cauda epididymal spermatozoa of mice (6) treated with NaF.

Underwood (19) has reviewed enzyme changes in chronic fluorotic animals. Fluoride has been used to block protein phosphates and thereby to "freeze" the phosphorylation states of protein in sperm homogenates. In the present study, activity of acid phosphatase in spermatozoa was reduced significantly throughout the treatment. NaF toxicity involves inhibition of spermatozoa was reduced significantly throughout the treatment. NaF toxicity involves inhibition of enzyme activities, particularly those in which divalent metal cations act as cofactors (20). Hence it is probable that the alterations in ATPase, SDH, and ACP in spermatozoa might be due to the fact that they are either Mg^{+2} , Ca^{+2} , or Zn^{+2} metallo-proteins.

Epididymal proteins are important as sperm antigens and for sperm viability (21). Therefore, a change in sperm protein might alter their motility and fertilizability as in the present study. According to McIvor *et al.* (22), fluoride is known to produce a marked K^+ efflux from intact cells. In the present study also, a significant decrease in the Na^+ and K^+ levels of the sperm was observed. This decrease might be due to the active K^+ efflux, disturbing the electrolyte balance and thereby reducing sperm motility. The above-mentioned alterations in sperm motility and metabolism might be the outcome of altered and hostile internal milieu of the epididymis of NaF-treated rabbits, since it is known that normal epididymal structure and its internal microenvironment are important for sperm maturation and for maintaining them in a viable, motile state (21,23). The present findings are in agreement with those of others (4,5). The reduction in sperm motility, sperm count and changes in their metabolism led to the significant decline in fertility of treated rabbits. Similar loss of fertility in NaF-treated male mice and rats has also been reported (5). Another factor leading to reduction of fertility may be due to the large number of deflagellated spermatozoa as well as their acrosomal, mid-piece and tail abnormalities, as observed by the modified silver-nitrate staining technique (10).

The withdrawal-of-treatment groups studied were: Withdrawal of NaF treatment alone for a month (Group IV), Withdrawal of treatment as in Group IV but fed vitamin C (Group V), and in Group VI calcium was fed during the withdrawal period. In Group IV virtually none of the induced effects was recovered with withdrawal of treatment. However, significant recovery was more pronounced with ascorbic acid than with calcium. It is known that ascorbic acid and its free radical monodehydroascorbic acid (MDHA) was potent reducing agents which activate several oxido-reduction reactions in tissues and have beneficial effects in drug-treated animals (24). Yu and Hwang (25) have also reported that high intake of vitamin C mitigated the effects of fluoride in mice.

The therapeutic effects of calcium against fluoride have also been long known. Narasinga Rao *et al.* (26) have studied calcium turnover in endemic fluorosis and reported its mitigating influence after calcium ingestion in fluoride poisoning in rats. Calcium has an important role to play in epididymal and vas deferens smooth muscle contraction (27), permeability of cell membranes and capillaries, sperm motility, interaction with cAMP (28), and activation of enzymes like APTase and succinate dehydrogenase. Hence, in the present study, when calcium was administered to rabbits during the withdrawal period, significant recovery in most of the induced effects was observed.

In Group VII animals, combined treatment of ascorbic acid + Ca^{+2} was found to result in greater recovery of all parameters affected by NaF than by individual administration of AA or Ca^{+2} . The recovery in the activities of ATPase and SDH is important, since these enzymes are involved in energy-releasing reactions and in oxidative metabolic processes of the sperm. Acid phosphatase is a lysosomal enzyme that has been in association with human acrosome. Recovery in this enzyme as well as protein concentration and Na^+ and K^+ levels of the spermatozoa caused normalization in their structure and functions.

It was observed that rats treated with NaF + AA or NaF + Ca^{+2} for 30 days also revealed recovery in several parameters of reproductive and

non-reproductive tissues compared to those treated with NaF alone (29,30). It is therefore suggested that the effects of administration of combination of ascorbic acid and calcium might be highly beneficial in the recovery of fluoride-treated animals. This has a direct bearing on ameliorating the sufferings of fluorosis-afflicted humans also, since they are incapable of synthesizing vitamin C. Such studies are now underway.

Acknowledgement

One of the authors (E.S.) is grateful to St. Theresa's College for Women, Eluru, for granting the necessary permission and financial assistance to carry out the doctoral work.

References

1. Susheela, A.K.: **Fluoride Toxicity**. Proceedings of the 13th Conference of the International Society for Fluoride Research. November 13-17, 1983, ISFR, New Delhi, 1985.
2. Tao, S. and Suttie, J.W.: Evidence for a Lack of an Effect of Dietary Fluoride Level on Reproduction in Mice. **J. Nutr.**, 106:1115-1122, 1976.
3. Messer, H.H., Armstrong, W.D. and Singer, L.: Influence of Fluoride Intake on Reproduction in Mice. **J. Nutr.**, 103:1319-1326, 1973.
4. Chinoy, N.J. and Sequeira, E.: Effects of Fluoride on the Histoarchitecture on Reproductive Organs of the Male Mouse. **Reprod. Toxicol.**, 3:261-268, 1989.
5. Chinoy, N.J. and Sequeira, E.: Reversible Fluoride Induced Fertility Impairment in Male Mice. **Fluoride**, [in press].
6. Chinoy, N.J. and Sequeira, E.: Fluoride Induced Biochemical Changes in Reproductive Organs of Male Mice. **Fluoride**, 22:78-85, 1989.
7. Chinoy, N.J., Rao, M.V., Narayana, M.V. and Keelakanta, E.: Microdose Vasal Injection of Sodium Fluoride in the Rat. **Reprod. Toxicol.**, [Comm.], 1990.
8. Chinoy, N.J., Rao, M.V. and Verma, R.J.: Micropuncture Techniques in Studying Microenvironment of Epididymis. **Indian J. Med. Res.**, 86:410-411, 1987.
9. Presad, M.R.N., Chinoy, N.J. and Kadam, K.M.: Changes in Succinic Dehydrogenase Levels in Rat Epididymis under Normal and Altered Physiological Conditions. **Fertil. Steril.**, 23:186-190, 1972.
10. Chinoy, N.J., Ranga, G.M., Highland, H.N. D'Souza, K.J. and Sequeira, E.: A Modified Method for the Differential Staining of Spermatozoa Using Alcoholic Acidic Silver Nitrate. **Int. J. Fertil.** [in press], 1990.
11. Quinn, P.J. and White, I.G.: Distribution of Adenosine Phosphatase Activity in Ram and Bull Spermatozoa. **J. Reprod. Fertil.**, 15:449-452, 1968.
12. Beatty, C.H., Basinger, G.M., Dully, C.C. and Bocek, R.M.: Comparison of Red and White Voluntary Skeletal Muscle of Several Species of Primates. **J. Histochem. Cytochem.**, 14:590-600, 1966.
13. Bessey, O.A., Lowry, O.H. and Brock, M.J.: A Method for the Rapid Determination of Acid and Alkaline Phosphatase with 5 Cubic mm of Serum. **J. Biol. Chem.**, 164:321-329, 1946.
14. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J.: Protein Measurement with Folin Phenol Reagent. **J. of Biochemistry**, 193:265-275, 1951.
15. Dean, N.A.: **Flame Photometry**. McGraw-Hill Book Co., Inc., 1960, p. 295.

16. Saralukumari, D., Varadacharyulu, N.Ch. and Ramakrishna Rao, P.: Effect of Fluoride Toxicity on Growth and Lipids in Liver, Kidney and Serum in Rats. **Arogya - J. Health Sci.**, 14:24-29, 1988.
17. Schoff, P.K. and Lardy, H.A.: Effects of Fluoride and Caffeine on the Metabolism and Motility of Ejaculated Bovine Spermatozoa. **Biology of Reproduction**, 1037-1046, 1987.
18. Blum, J.J. and Hayes, A.: Specific Anion Effects on ATPase Activity, Calmodulin Sensitivity and Solubilization of Dynein ATPase. **J. Cell Biochem.**, 25:197-212, 1984.
19. Underwood, E.J., (Ed.): **In: Trace Elements in Human and Animal Nutrition**. Academic Press, New York, Sanfrancisco, London, 1977.
20. Hodge, H.C. and Smith, F.A.: **Fluoride in Metallic Contaminants and Human Health**. Lee, D.H.K. (Ed.), Academic Press, New York, 1972, p. 163.
21. Chinoy, N.J.: Structure and Function of Epididymis in Relation to Vulnerable Points of Intervention for Male Fertility Regulation. **Indian Review of Life Sciences**, 4:37-68, 1984.
22. McIvor, M.E., Cummings, C.C., Mower, M.M., Baltazar, R.F., Wenk, R.E., Lustgarten, J.A. and Solomon, J.: The Manipulation of Potassium Efflux during Fluoride Intoxication: Implication for Therapy. **Toxicology**, 37:233-239, 1985.
23. Prasad, M.R.N. and Rajalakshmi, M.: Recent Advances in the Control of Male Reproductive Functions. **In: Greep, R.O. (Ed.): International Reviews of Physiology, Reproductive Physiology II**. University Park Press, Baltimore, MD, USA, 13:153-199, 1977.
24. Chinoy, N.J.: Ascorbic Acid Turnover in Animals and Human Tissue. **J. Anim. Morphol. Physiol.**, Silver Jubilee Vol.: 68-85, 1978.
25. Yu, M.H. and Hwang, H.L.S.: Influence of Protein and Ascorbic Acid on Fluoride-induced Changes in Blood Composition and Skeletal Fluoride Deposition in Mice. **In: Tsunoda, H. and Yu, M.H. (Eds.): Fluoride Research, 1985, Studies in Environmental Science**. Vol. 27., Elsevier/Amsterdam, p. 203-210.
26. Narasinga Rao, B.S., Krishnamachari, K.A.V.R. and Vijayasarthi, C.: ^{47}Ca Turnover in Endemic Fluorosis and Endemic Genu Valgum. **Brit. J. Nutr.**, 41:7-14, 1979.
27. Chinoy, N.J. and Chinoy, M.R.: Differential Contractile Response of Normal Vas Deferens in Rodents in Correlation to Their Calcium and Electrolyte Levels. **J. Biosci.**, 5:173-180, 1983.
28. Rasmussen, H.: The Cycling of Calcium as an Intracellular Messenger. **Scientific American**, October: 66-73, 1989.
29. Sharma, M.: **Effects of Ascorbic Acid and Calcium on Some Vital Organs of Sodium Fluoride Ingested Male Rats**. M. Phil. Dissertation, Department of Zoology, School of Sciences, Gujarat University, Ahmedabad, India, 1990.
30. Reddy, V.V.V.S.N.G.L.P.C.: **Effects of Ascorbic Acid and Calcium on Reproductive Functions of Sodium Fluoride Ingested Prepubertal Male Rats**. M. Phil. Dissertation, Department of Zoology, School of Sciences, Gujarat University, Ahmedabad, India, 1990.
