

# QUALITATIVE CHANGES IN THE OVARIAN HISTOARCHITECTURE OF SEXUALLY MATURE CYCLING FEMALES OF SWISS ALBINO MICE CHALLENGED BY VARIOUS DOSES OF SODIUM FLUORIDE

**\*Manisha Mathur**

*Department of Zoology, G.N. Khalsa College, Matunga Mumbai-19, (India)*

## ABSTRACT

*Adult female mice (B.Wt.21.5 gms )were treated at a 5mg/kg BW dose and 10 mg / kgBWt of sodium Fluoride for five days in a drinking water. Control animals were given equal dose of deiononised water .Qualitative changes were studied in ovary. Histopathological changes in primary, secondary, Graffian and Atretic follicles as changes in unilaminar, bilaminar and multilaminar follicles were observed and changes in no. of granulose cells, etc. were evaluated. Significant qualitative changes were noticed. These changes were characterised by disorganization. Necrosis, devacuolization and Infiltration very prominently in the follicles.*

**Key Words:** *Fluoride, Toxicity, Ovary, Histopathology , Albino Mice.*

## I. INTRODUCTION

Titers of estrogens and progestens synchronise and regulate many structural, physiological, and biochemical changes in the fallopian tube (= oviduct) and uterus, which create a milieu interior in them to facilitate gamete transport, nutrition of sex cells, events before and during fertilization, and cleavage of zygote upto the blastocyte stage. Implantation of blastocyte on the endometrium of the uterus marks the culmination of these pre-gestational events [1]. Progesterons and estrogen are important in initiating and sustaining sexual behaviour and also provide hormonal support to the series of extremely complex processes during gestation [2].

Fluoride forms one of the source of toxicants in ground water. Their concentration in numerous states of India reaches beyond the permissible limits of 1.0 ppm[3]. Fluoride toxication is a global problem. The resulting disorder i.e., fluorosis is known to cause a variety of syndromes that affect neural, renal, cardiovascular, muscular and skeletal structures and functions [4,9]. Relatively little is known about the affect of sub-lethal doses of sodium fluoride on the ovarian histoarchitecture in placentals. Studies on the ovary of rabbit indicated that the gametogenic functions are disturbed in response to imbibition of fluoride in drinking water [5,8]. Except this limited information, other reports are fragmentary and are debatable [6,7] There is thus considerable lacuna in our understanding on the effects of fluoride toxication in case of mammalian ovary.

Using sexually mature cycling females of Swiss albino mice as a model, different sub-lethal doses (5 mg and 10 mg/kg BW) of sodium fluoride were tested with a view to delineate the qualitative and quantitative changes in the ovarian histometrics.

## **II. MATERIALS AND METHODS**

Laboratory bred cycling adult females of Swiss albino weighing 21.5 0.5 gm to 25.5 0.5 gm were used as a 'model' in the present study.

### **A) Experimental protocol**

Two groups of mice ( $n = S$  in each group) were given sodium fluoride at a dose level of 5 mgs/kg body weight and 10 mgs/kg body weight for 5 days. The control group of mice were given deionised water for 5 days. Sodium fluoride (to experimental group of mice) was given orally through drinking water. However; both groups had ad-libitum access to standard rodent chow. The animals were maintained in plastic cages with wire mesh-nettings. They exposed to standard photoperiodic conditions of laboratory (12 h of light and 12 h of dark). The weight of the animals before and after the experiments was recorded. All experiments were run in triplicate with control.

### **B) Surgical procedure**

Animals of experimental and control group were sacrificed on day 6 of treatment by cervical dislocation. The ovaries were dissected out surgically under semi-sterile conditions. Excess fascia and blood clots was removed. They were weighed individually on a monopan balance.

### **C) Processing of tissues**

The contralateral ovaries of experimental and control group of mice were fixed in aqueous Bouin's fluid for 2 hours. They were dehydrated in graded ETOH series, cleared in xylene; infiltrated with and embedded in pure filtered paraffin wax (m.p. 58°C). Deparaffinised sections (5-7) were stained by haematoxylin-Eosin, and Geimsa to monitor the extent of changes in the ovarian histoarchitecture. Every alternate section of the contralateral ovary was microscopically examined and appropriate areas were microphotographed and enlarged. Stages in follicular atresia were also microphotographed to record the vulnerability of each follicle type to fluoride toxication.

### **D) Parameters of evaluation**

The classification of follicles was done according to the method of Pederson and Peters (1968). The number of granulosa cells investing each follicle type, and number of granulosa cell layers were principally used in the identification of follicle types. The population of each follicle type was also quantitated and the number of corpora lutea in contralateral ovaries were counted. Atretic Follicles were identified on the basis of desquamation of granulosa cells, nuclear pycnosis, chromatolysis, and loss of shape.

## **III. OBSERVATIONS AND RESULTS**

In the present study the affects of chronic (5 days ) oral ingestion of sodium fluoride (5 mgs/kg body weight and 10 mgs/kg body weight) on the morphometrics (weight and external appearance) and histoarchitecture of the

ovary of cycling females of Swiss albino mice were monitored and compared with control ( on the sixth day). It was observed that varying degrees of structural aberrations occurred which commensurate with the numerical changes produced in the ovaries of treated mice vis-a-vis control.

**(I) Qualitative changes**

**(a) Control**

Marked difference in number of granulosa cells and cell layers of these follicles was observed clearly. Follicular atresia also occurred but was not very well marked in control animals.

**(b) Histoarchitecture alteration in ovaries after chronic (5 days) oral ingestion of 5 mgs/kg body weight of sodium fluoride.**

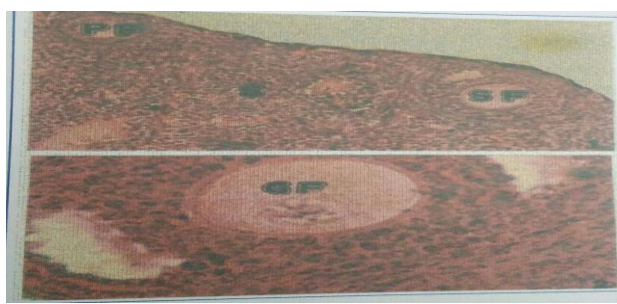
The overall shape of the ovaries was not altered nor there was any significant change in the weight of this organ (P 0.95%) as compared with control. The multilaminar and Graffian follicles were most effected. These follicles began to degenerate along with haemorrhage in blood capillaries but most of the follicles reach the maturity. The total number of atretic follicle was hence found to be high as compared with control.

**(c) Histoarchitecture alterations in ovaries after chronic (5 days) oral ingestions of (10 mgs/kg body weight) NaF**

The overall shape of the ovaries was not altered nor there was any significant (1.99%) change in organ weight as compared with control.

Five days chronic ingestion of (10 mgs/kg body weight) NaF revealed marked changes in ovarian histoarchitecture showing pronounced degeneration.

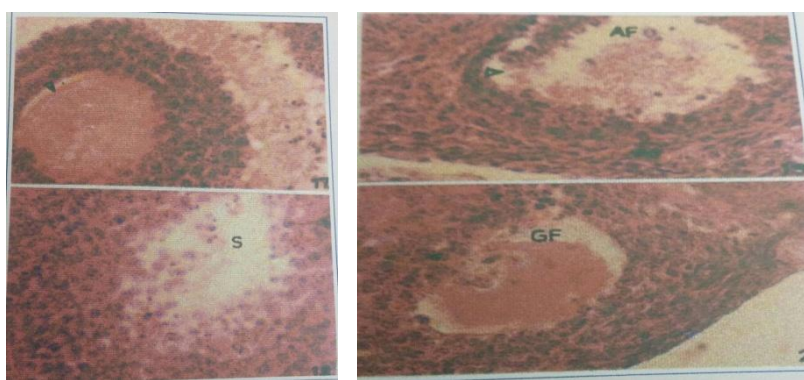
The main sequence of atretic changes involved nuclear degeneration characterised by chromatolysis, rupture and dissolution of nuclear membrane. Granulosa cells associated with degenerating follicle types (bilaminar, multilaminar and Graaffian follicle) showed desquamation, cytolysis and nuclear dissolution. Extensive vacuolation also occurred in these follicles. Only few follicles reach the maturity.



**Fig:1 - HIGH POWER PHOTOMICROGRAPH SHOWING GRAFFIAN AND ATRETIC FOLLICLES IN A CONTROL OVARY(HAEMATOXYLIN EOSIN STAINING)**



**FIG 2&3: HIGH POWER PHOTOMICROGRAPH SHOWING GRAFFIAN AND ATRETIC FOLLICLES IN A CONTROL OVARY(HAEMATOXILIN EOSIN STAINING)**



**Fig:4&5-HIGH POWER PHOTOMICROGRAPH SHOWING GRAFFIAN AND ATRETIC FOLLICLES IN A CONTROL OVARY(HAEMATOXILIN EOSIN STAINING)**

#### **IV.DISCUSSION**

Sub lethal doses of NaF (5 mgs/kg weight) administered to post pubertal, cyclic females of Swiss Albino mice exhibited marked alterations in the cytoarchitecture of ovarian cell type.

The bi and trilaminar follicles showed shrinkage in the Ooagk and swelling of nucleus after day 1 of treatment. Desquamation of granulosa cells, invasion of follicular fluid were observed in the multilaminar and prenatal follicles after day 1 of NaF treatment. Significant alterations were not observed in a diameter of the follicles of oocytes after day 1 of treatment, type b atretic follicles showed slight increase in their population after day 1 of treatment while the others showed no significant alterations.

The bilaminar follicles showed Haryolysis of nucleolus after day 5 of treatment.

The dissolution of the oocyte were observed in bilaminar follicles after day 5 of NaF treatment. Invasion of the Oocyte by the granulosa cells were observed in the tri and multilaminar follicles after 5th day of treatment. The disappearance of antral fluid of antral follicles and loosening and shedding of the granulosa cells of cumulus oophorus of mature graffian follicles were observed after day 5 of NaF treatment.

The Oocytes and granulosa cells of all the follicles were degeneration and the follicles lost its contour on day 6 of administration of NaF.

Qualitative changes which occur in ovary after NaF ingestion include, shrinkage in ova and swelling of nucleus. Desegmentation of granulosa cells was also observed in trilaminar, multilaminar, graffian follicles, preantral and atretic follicle even after ingestion of 5 mg/kg NaF.

After increasing the dose to 10 mg/kg BW description of the granulosa cells and cell layers, appearance of large antra spaces, filled with antral fluid, large blood vessels, pyknosis in granulosa etc. was observed. Invasion of an oocyte by the granulosa cells, in tri and multilaminar follicles, disappearance of antral fluid in antral follicles, loss of cells in mature graffian follicles and loss the contour of the follicles, accumulation of degenerating cells in follicular cavity, as well as vacuolization of corpus luteum size of follicles were the characteristic observation.

Histoarchitecture study carried out in the present study thus suggest that chronic ingestion of sodium fluoride even for a short period induces deleterious changes in female reproductive structures.

#### **V.ACKNOWLEDGEMENT**

I dedicate my work to my Father Mr.K.G.Mathur, Mother Amar Mathur, My Brothers Praseon Mathur and Arvind mathur and my Bhabhies who are my backbone. Without their blessings my work is never possible. I acknowledge my heartiest feelings for the sacrifice of my husband Sanjay and my children Kartik, Gaurang, Mohit and Rimjhim during my study.

#### **REFERENCES**

- [1.] **Guraya (1985).** *Biology of Ovarian Follicles in Mammals*. Springer-Verlag, Berlin, 320. *Indian micro- and megachiropteran Mammals*. Unpubl. Ph.D. Thesis, University of Udaipur, 216
- [2.] **Jaroli D (1980).** Seasonal histoenzymological and histochemical alterations in the ovary and uterus of Indian micro and megachiropteran mammals. Unpubl. Ph.D. Thesis, University of Udaipur, 216. **Lall (1986).** Folliculogenesis in *Rhinopoma kinneari* Wroughton (Microchiroptera: Mammalia). *Myotis*, 23–24, 37–44.
- [3.] **R John Aitken and Shaun D, Roman (2009).** *Advances in Experimental Medicine and Biology* 636, 154–171.
- [4.] **Trivedi S 1991.** Seasonal histochemical and histoenzymological alterations in the ovary and uterus of certain chiroptera (Mammalia) of Rajasthan. Unpublished. Ph.D. Thesis. ML Sukhadia University, Udaipur, 227
- [5.] **Wimsatt WA 1979.** Reproductive asymmetry and unilateral pregnancy in Chiroptera. *Journal of Reproduction and Fertility* 56 345–357.
- [6.] **Mathur M.** Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online) An Online International Journal Available at <http://www.cibtech.org/jls.htm> 2012 Vol. 2 (2) April-June, pp.115–117.



- [7.] *Hess et al., 1958.* R Hess, D.G Scarpelli, A.G.E Pearse The cytochemical localization of oxidative enzymes. II. Pyridine nucleotide linked dehydrogenases. J. Biophys. Biochem. Cytol., 4 (1958), pp. 754-760. Ihse and Arnesjo, 1973. I Ihse, B Arnesjo The phospholipase A2 activity of human small intestinal contents. Acta Chem.
- [8.] *Nachlas et.al.. (1957a)* Histochemical demonstration of leucine aminopeptidase. Journal of Histochemistry and Cytochemistry 5:264. *Nachlas M.M., Tsou K.C., De Souza E. et al. (1957b)* Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted dite.
- [9.] Mathur M. International Journal Trends in Life Sciences ,ISSN 2327-5476,an International Journal ,Pg123-126 The effect of Chronic administration of fluoride on reproductive organs in female albino mice.