

# Effect of Fluoxetine on Epididymis of Albino Rats: A Histological Study

Alka Aggarwal, S L Jethani, R K Rohatgi, Juhi Kalra\*

**Abstract :** Fluoxetine is a prototype of Selective Serotonin Reuptake Inhibitors (SSRIs), with a plasma half-life of 2 days. Fluoxetine is widely and inadvertently used as an antidepressant for various psychiatric illnesses in high doses and for long duration. The aim of present study was to establish the fact that higher doses and long term use of Fluoxetine (SSRI) cause change in the histology of epididymis of albino rats. The present study was carried out on 32 adult male albino rats. Fluoxetine was administered intraperitoneally for 2 weeks, 4 weeks and 12 weeks with mild (10mg/kg/day), moderate (20mg/kg/day) and severe doses (40mg/kg/day). Histological slides were prepared and stained with H and E stain. On examination, Epithelial cell hypertrophy, Intracytoplasmic Vacuolations, intercellular vacuolations, reduced number of spermatozoa, sloughed testicular germ cells and cell debris in the Epididymal lumen were found in treated groups. These changes may cause infertility.

**Key words** – Epididymis, sperm maturation, sperm motility, Selective Serotonin Reuptake Inhibitors, Fluoxetine, Hypothalamo-pituitary-adrenal axis (HPA axis).

## 1. Introduction:

Fluoxetine is a long acting widely used antidepressant, prototype of Selective Serotonin Reuptake Inhibitors (SSRIs), with a plasma half-life of 2 days. Its active demethylated metabolite has a half-life of 7-10 days. It is used in children above 7 years and in adults on the basis of its efficacy and lower side effect profile [1]. Fluoxetine inhibits the 5-hydroxytryptamine (5-HT-Serotonin) reuptake. Increased synaptic availability of serotonin stimulates a large number of postsynaptic (5-HT) receptor subtypes which lead to complex secondary sexual responses in the form of loss of libido, delayed ejaculation, anorgasmia and impaired orgasm, decreased testicular development and decreased Sertoli cell population, which may lead to infertility in adults [2]. Depression may also be associated with sexual disturbances especially reduced libido [3]. Studies reported that Fluoxetine induces sister-chromatid exchanges (SCE's) in bone marrow cells and sperm abnormalities inducer. The response

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was dose-dependent, and the highest tested dose increase about two times SCE and four times the sperm abnormalities. The percentage of sperm count and sperm motility decreased with increase the dose [4, 5]. For psychiatric illnesses (Depression and anxiety) the SSRIs are used for a long duration. The aim of the present study was to see the effects of varying doses for different durations of fluoxetine administration on the histology of epididymis, and to verify the germinal cell destructive effects of Fluoxetine on the testis by evaluating the Epididymis of Wistar albino rats.

## 2. Materials and Methods

The study was done on 36 Albino rats (*Rattus norvegicus*, Wistar strain) of both sexes with an average weight of 120-150 grams. These animals were obtained from the Central Animal House of Himalayan Institute of Medical Sciences, Dehradun, after obtaining the prior approval of Institutional Animal Ethical Committee (IAEC). Before starting the study it was confirmed that all the rats were disease-free and healthy. All the groups of rats were housed separately in different cages in groups of 2 to 3 rats per cage. They were fed with standard balanced laboratory diet and were given access to food & water ad libitum. After 2-week acclimatization period in individual cages under a 12Light: 12Dark cycle this study was started.

This study was conducted in 3 Phases of 2, 4 and 12 weeks duration. Each phase comprised of 12 rats. These 12 rats were further subdivided into 4 groups (a Control and 3 Experimental groups) of 3 rats each. Group 1(Control) rats received equal volume of normal saline (vehicle) intraperitoneally. The Experimental groups (Group 2, Group 3 & Group 4) rats were injected with 10 mg/kg/day, 20 mg/kg/day and 40 mg/kg/day of Fluoxetine intraperitoneally respectively.

After completion of each of the three phases, the Epididymis was dissected out after sacrificing all the rats under ether anaesthesia. The tissues were fixed in 10% formalin, processed and blocks were made in paraffin wax. 4-5 µm thick sections were cut and stained with haematoxylin and eosin. The sections were examined in the light microscope under magnification (X100, X200). Student's t- test was used for statistical analysis.

**3. Observations:**

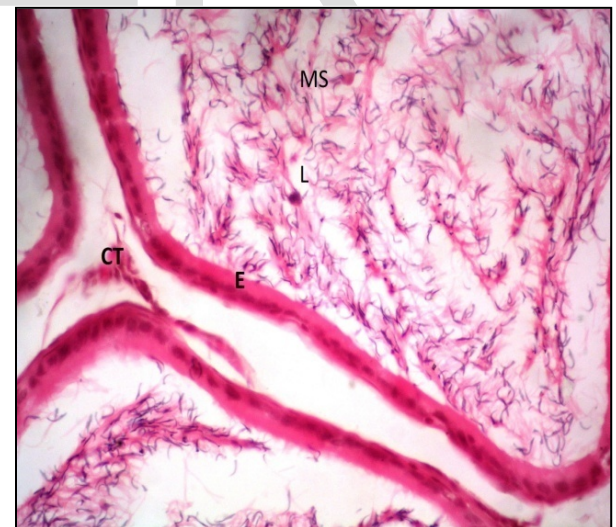
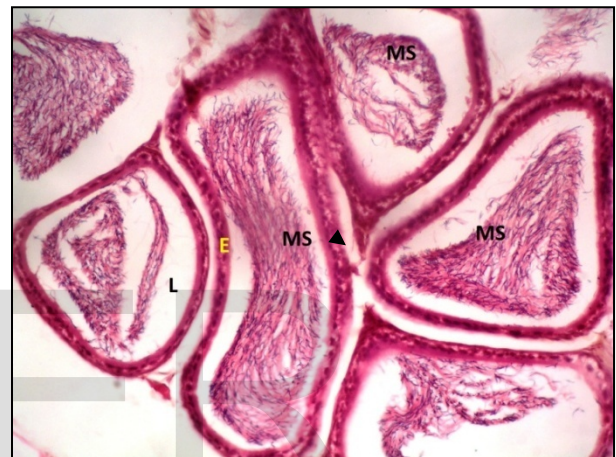
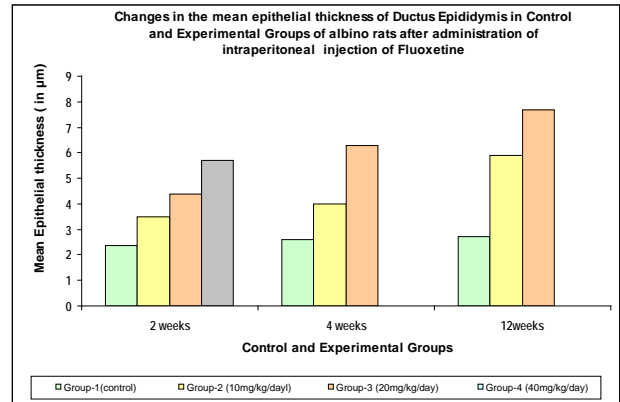
In control rats (Group 1) the lining epithelium of Epididymis was Pseudostratified columnar epithelium, composed of mainly tall columnar principal cells and triangular shaped basal cells & few halo cells (migrating lymphocytes) and light cells. The light cells of Epididymal epithelium may have a role in clearing the lumen. The columnar cells bear non motile processes (stereocilia). The nuclei of the cells were elongated and lay somewhat at different levels. The lumens of Epididymis were filled with spermatozoa. The epithelium was lined by a thin sheet of circular smooth muscle fibers. The interstitial tissue of the epididymis was composed of loose connective tissue, which contained fibroblasts, collagen fibers, abundant ground substance, and small blood vessels (Table 1, Graph 1, Fig. 1).

**Table 1**  
**Changes in the mean thickness (in µ) of the epithelium of Ductus Epididymis**

| Groups                   | 2weeks<br>(Mean ± SD)         | 4weeks<br>(Mean ± SD)  | 12weeks<br>(Mean ± SD)           |
|--------------------------|-------------------------------|------------------------|----------------------------------|
| Group 1<br>(Control)     | 2.38 ± 0.52                   | 2.6 ± 0.49             | 2.7 ± 0.46                       |
| Group 2<br>(10mg/kg/day) | 3.50 ± 0.71<br>P=0.003        | 4.00 ± 0.89<br>P=0.003 | 5.90 ± 1.51<br>P=0.0000053       |
| Group 3<br>(20mg/kg/day) | 4.4 ± 0.52<br>P=0.0002        | 51.9 ± 7.09<br>P=0.01  | 7.70 ± 0.46<br>P=0.0000000000045 |
| Group 4<br>(40mg/kg/day) | 5.70 ± 0.48<br>P=0.0000000001 | Animals died           | Animals died                     |

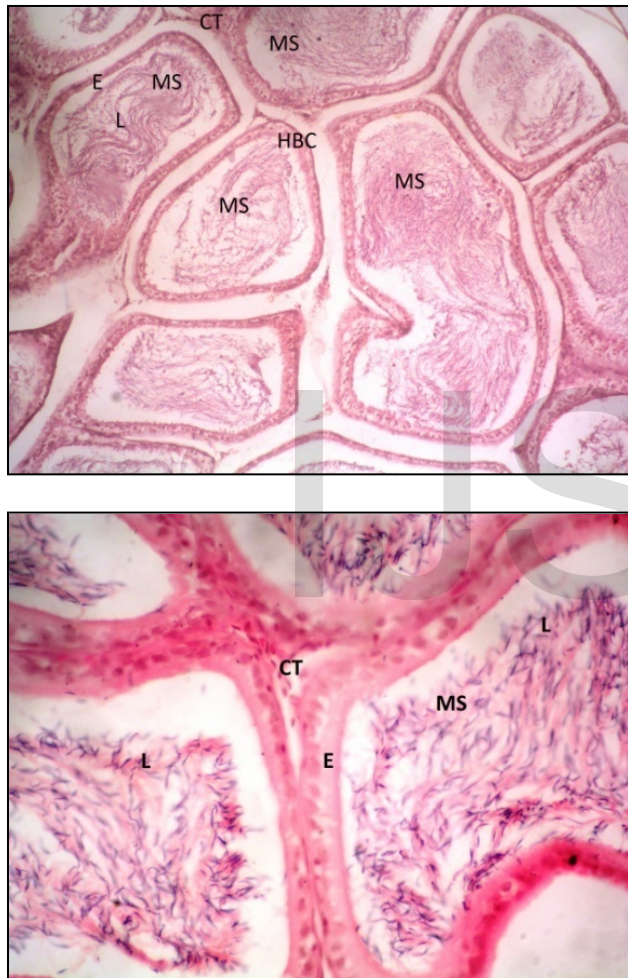
(P < 0.01 – Highly significant, P < 0.05 – Significant, P > 0.05 – Non significant)

**Graph -1**



**Fig1: Photomicrograph of the epithelium (E) of Ductus Epididymis in Control (Group 1) of albino rats showing normal pseudostratified columnar epithelium(E), lumen (L) filled with mature sperms (MS) H & E; X 100, X200.**

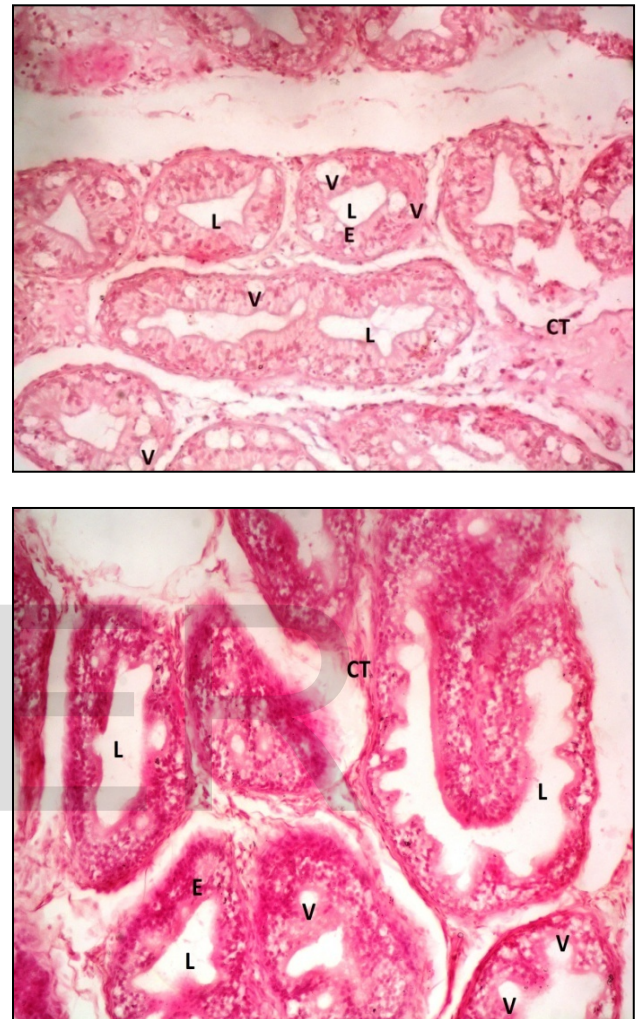
It was observed that Experimental rats (Group 2) which were administered Fluoxetine (10mg/kg/day) showed a normal histological appearance of ductus epididymis & interstitial tissue in phase I & II. The pseudostratified columnar epididymal epithelium increased in the height in comparison to Control. The lumen of the epididymis contained numerous spermatozoa which were closely packed in the wide lumen of the ductus epididymis. Phase III rats showed appearance of small intracytoplasmic vacuolation (Table 1, Graph 1, Fig. 2).



**Fig 2: Photomicrograph of the Ductus Epididymis in Phase III, Group 2 Experimental rats showing appearance of intracytoplasmic vacuolation (V), lumen filled with mature sperms (MS) H & E; X 100, X200.**

Group 3 Experimental rats (20mg/kg/day) phase I and II also showed a normal histological appearance except increase in epithelial cell height. Phase II rats showed appearance of intracytoplasmic epithelial vacuolations. Phase III showed both intercytoplasmic and intercellular vacuolations, decrease in the diameter of ductus

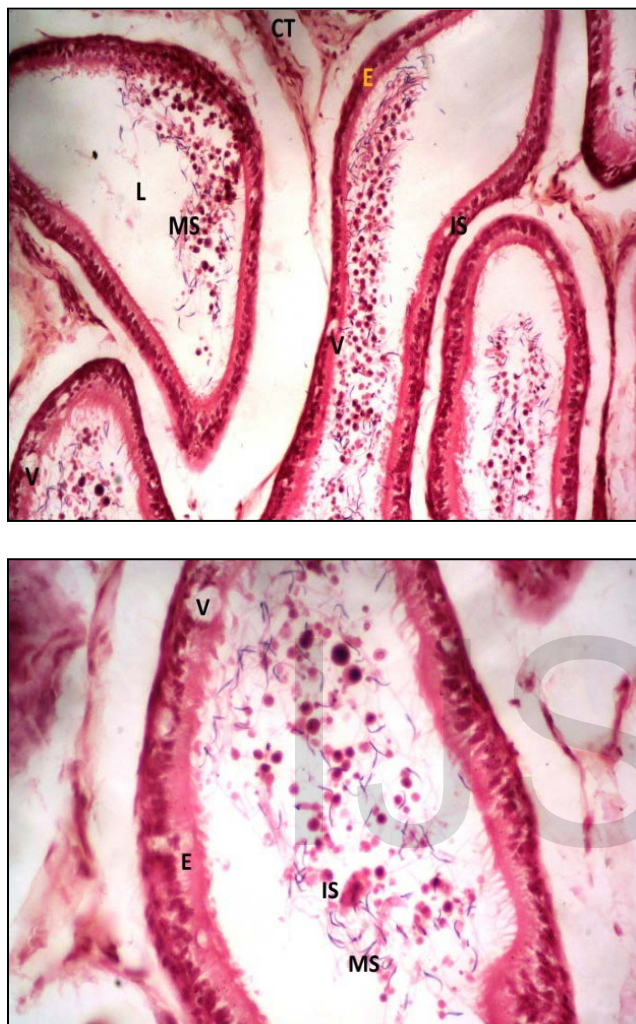
epididymis with irregular small lumen (papillary appearance) and absence of spermatozoa in the lumen. The stereocilia appear apparently decreased. (Table 1, Graph 1, Fig. 3). The affinity to eosin was apparently decreased.



**Fig 3: Photomicrograph of the Ductus Epididymis in Phase III, Group 3 Experimental rats showing prominence of vacuolation (V) (both intracellular & intercellular) with hypertrophy of epithelial cells (E), lumen (L) appears narrow and irregular, with absence of sperms in lumen H & E; X 100, X200.**

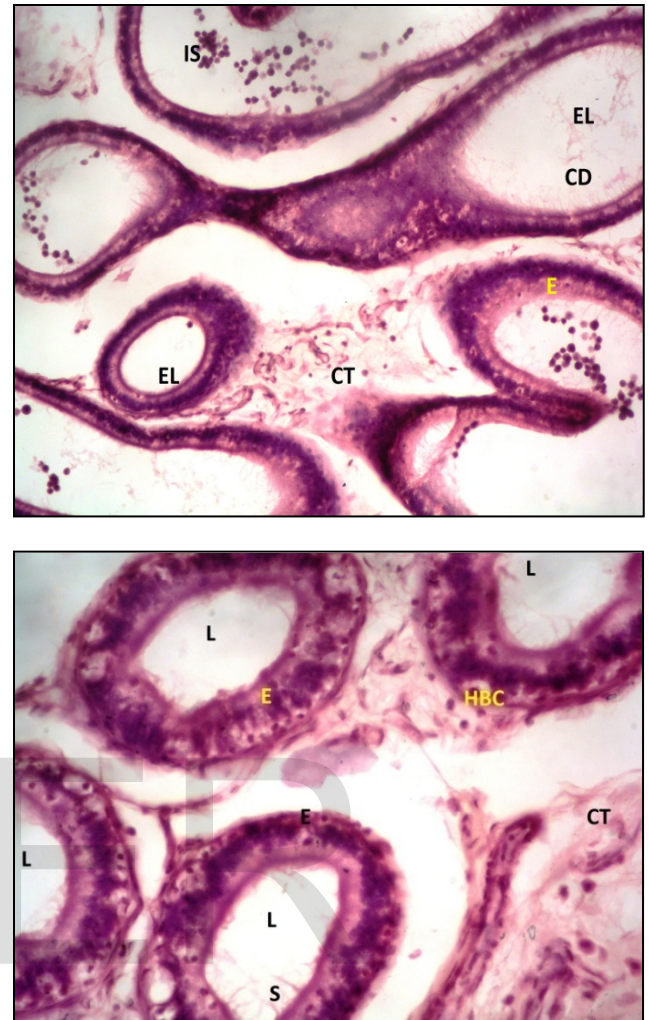
Some Experimental rats of Group 4 (40mg/kg/day) which were sacrificed on 7<sup>th</sup> day due to ill health showed that the lumen was filled with small rounded cell bodies, scanty normal sperms and debris, which appeared to be derived from germ cells. The diameter of Ductus Epididymis was also decreased. Epithelium showed changes in character as evidenced by increased affinity for stain. The spermatozoa almost lost their morphological features. The lumens of epididymis were filled with round masses

of cells which appeared denatured proteinaceous material of spermatozoa. The stereocilia appear normal. (Fig.4).



**Fig 4: Photomicrograph of the Ductus Epididymis in Phase I, Group 4 Experimental rats which received the drug for 7 days showing the presence of rounded immature sperms (IS) with cell debris (CD) with some normal mature sperms (MS) in the lumen(L), hypertrophy of epithelial cells(E) with prominent stereocilia, and presence of vacuolation (V). H & E; X100, X 200.**

Survived Experimental rats of phase I, Group 4 (40mg/kg/day) had a mean thickness of epithelium  $5.70 \pm 0.48 \mu$ . All the values were found to be highly significant on application of Student's t-Test ( $p < 0.01$ ). Diameter of tubules of epididymis was reduced, lumen was empty. Sterocilia appear apparently prominent. (Table1, Graph1, Fig. 5)



**Fig 5: Photomicrograph of the Ductus Epididymis in Phase I, Group 4 Experimental rats which received the drug for 2weeks showing the presence of rounded immature sperms (IS) with cell debris (CD) within the lumen(L), hypertrophy of epithelial cells with prominent stereocilia(S). Vacuolation (V) (both intracellular & intercellular), and hypertrophied basal cells(HBC). The tissues have taken deep stain. H & E; X 100, X200.**

Observations of rats of all the three phases on different doses of Fluoxetine showed increased thickness of epithelium of ductus epididymis (Table1, Graph1 & Fig.1-5). The most conspicuous observation noted in this study was that most of the Experimental rats of Group 4 (40 mg/kg/day) developed muscle twitchings, movements became sluggish and passed loose stools after about 2 weeks of treatment. All the animals died during the 3<sup>rd</sup> week of administration of the drug. Perhaps, this was a toxic dose to these animals (Table 1).

#### 4. Discussion:

Spermatozoa leaving the testis and entering the long convoluted tubule known as the epididymis are non-functional gametes. It is only during transit through the epididymis that spermatozoa undergo maturation and acquire progressive motility and the ability to fertilize ova. Maturation involves the interaction of spermatozoa with proteins that are synthesized and secreted in the epididymal epithelium.

The importance of understanding epididymal function and sperm maturation is emphasized by the fact that up to 40% of infertile men exhibit idiopathic infertility that may reflect sperm maturational disorders [6].

Previous studies reported that epididymis contains functional LH receptor. Z M Lei et al reaffirm that androgens and LH are important for normal epididymal morphology and function [7].

The Hypothalamo-Pituitary-Adrenal (HPA) axis is involved in the mood disorders, anxiety disorder, bipolar disorder, insomnia, posttraumatic stress disorder. Antidepressants, serve to regulate HPA axis function. The pharmacological elevation of serotonin level in hypothalamus by Fluoxetine cause activation of HPA axis. The paraventricular nucleus of the hypothalamus, secrete vasopressin and corticotropin-releasing hormone (CRH). CRH regulate the anterior lobe of the pituitary gland, and vasopressin stimulate the secretion of adrenocorticotrophic hormone (ACTH), once known as **corticotropin**. ACTH in turn acts on the adrenal cortex, which produces glucocorticoid hormones (mainly cortisol in humans). Glucocorticoids in turn act back on the hypothalamus and pituitary (to suppress CRH and ACTH production) in a negative feedback cycle. This leads to decrease secretion of gonadotrophic hormones (LH and FSH) which are essential for spermatogenesis and steroidogenesis. In the periphery, excessive free serotonin continuously stimulates the smooth muscle of blood vessels directly via 5-HT<sub>2R</sub> or indirectly via Thromboxane A<sub>2</sub>. This action might induce vasoconstriction and smooth muscle proliferation, contributing to microcirculation disturbances, leading to a decrease in blood supply, fibrosis and atrophy of Leydig cells. Leydig cell 5-HT<sub>2R</sub> stimulation by serotonin may induce the corticotrophin releasing factor (CRF). It has a negative effect on the HPG axis. CRF also exerts a local inhibitory role on androgen secretion of LH related interstitial cells [8, 9].

Decreased plasma testosterone and LH levels alters metabolic activities of cells of epididymis which is responsible for changes in character of cells and diameter of epididymis. Fluoxetine caused marked suppression of spermatogenesis via elevating the level of serotonin. Serotonin induces suppression of hypothalamus-pituitary-testis axis mediated by activated hypothalamus-pituitary-adrenocortical axis resulting in fall of plasma LH, FSH

and testosterone levels. Testosterone and FSH act directly upon germinal epithelium. The number of spermatozoa was greatly reduced. The lack of spermatozoa in present study seems most likely due to the decreased production by the testis, an effect of fall in plasma testosterone levels.

Drug information literature of SARAFEM also reported epithelial cell hypertrophy, epididymal vacuolation and hypospermia in experimental rats which received the highest dose (30mg/kg/day) of Fluoxetine orally. Observations of this study were found to be similar to the above study [10].

Martin-Du Pan RC et al reported that Environmental factors such as alcohol or drugs represented 12% of the etiologies for infertility in adult males (11). They also reported that among the SSRIs, Fluoxetine was the commonest drug which caused infertility in males by decreasing the sperm count and motility [12].

Taylor et al examined the effects of chronic fluoxetine administered 0.75 mg/kg of body weight/day intraperitoneally for 4 weeks on the reproductive system of adult male Long Evans rats and reported no change in Serum testosterone level and no histopathological changes in the Adrenals, Epididymis, Testes, Penis, Seminal Vesicles, Bulbospongiosus muscles and ventral Prostate [13]. Their findings were also similar to findings of our study at low doses.

HN Bataineh reported the decreased sperm motility and density in testes and cauda epididymides of long term treated Sprague-Dawley adult male rats with fluoxetine (200mg/kg/day) for 60 days [14]. Studies suggest that testes and epididymis of normal adult rats have very consistent morphology and have very little abnormalities. The presence of sloughed testicular germ cells and cell debris in the epididymal lumen is a very sensitive indicator of disrupted spermatogenesis in the testis. Subtle disturbances of spermatogenesis are often more readily identified by changes in epididymal contents than by the testicular changes [15].

#### 5. Conclusion:

The present histological study results confirmed the previous reports about Fluoxetine induced male infertility and sexual disorders. In the present scenario every person of any age group and sex may suffer or pass from a period of anxiety and depression and therefore needs a prescription for use of these drugs. There is an essential need to know their safe dose and duration. A further research is essential to know whether these changes in the male genital system are reversible or irreversible. Clinicians must take precautions in prescribing the dose and duration of Fluoxetine to their patients.

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