# Effects of long-term use of fluoxetine on fertility parameters in adult male rats

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**Abstract**

The effects of long-term ingestion of fluoxetine on fertility were investigated in Sprague-Dawley male rats. Adult male rats were exposed to fluoxetine at a concentration of 200 mg/kg for 60 days.

Long-term ingestion of fluoxetine for 60 days caused a great decrease in spermatogenesis in seminiferous tubules of the testes. Sperm motility and density were also significantly reduced in cauda epididymides and testes of the treated group. The weights of reproductive organs (testes, epididymides, ventral prostrate and seminal vesicle) were decreased considerably. The hormonal assay also showed significant decrease in testosterone levels and FSH levels. Testicular cell population dynamics also demonstrated a decrease in the number of both primary and secondary spermatocystes and spermatids in the treatment group. The number of female rats impregnated by male rats on long-term fluoxetine diet had decreased. The number of implantations and the number of viable fetuses were also notably decreased in female rats impregnated by male rats ingested fluoxetine. Fluoxetine caused a slight decrease in body weight, when initial and final body weights were compared in the experimental groups. Levels of ALT and AST were found to be significantly increased in the treated group when compared to the control. Histometry of reproductive organs confirmed these results.

In conclusion, these results confirm that the long-term fluoxetine ingestion produce adverse effects on fertility and reproductive system in adult male rat. Thus, it would be of great interest to investigate the impact via long –term treatment with fluoxetine in male human fertility.

ORIGINAL ARTICLE

## INTRODUCTION

Fluoxetine is an antidepressant drug that is widely-prescribed, has been described as a selective 5-hydroxytryptamine (5-HT) uptake inhibitor (Wong *et al.*, 1995). However there is evidence that it can also inhibit norepinephrine while FSH levels were not notably changed uptake in brain (Stanford, 1996). 5-HT re-uptake inhibitor drugs have been associated with male and female sexual side effects. While there are reports indicating that fluoxetine improve sexual behavior (Power-Smith, 1994), evidence also exists indicating a link between fluoxetine treatment and sexual dysfunction (Shen and Hsu, 1995). In addition, fluoxetine causes alterations in sexual behaviors in rats (increased intermount-bout intervals, time-outs, grooming time, ejaculation latency, number of mounts per mount bout, and number of mount bouts per ejaculation (Yells *et al.*, 1995).

It is well known that clinical disorders of emission and ejaculation can be the consequence of psychogenic disease and tricyclic antidepressant drugs have been clinically used to treat these disorders (Benson, 1994). The male reproductive tract is under adrenergic neuronal influence (Benson, 1994), so the clinical beneficial effect of these drugs could be attributed to their effects in norepinephrine contents in CNS.

The mechanism for the testicular toxicity of fluoxetine could be explained by the decreased in the testosterone levels. This reduction appears to be mediated through the central nervous system (Treinen and Chapin, 1991; Anderson *et al.*, 1992). However, it is unlikely that hormone changes can explain the atrophy, since it has been shown that spermatogenesis can be maintained in the presence of significantly reduced intratesticular testosterone (Rommerts *et al.*, 1988; Zirkin *et al.*, 1989). Thus, these studies suggest that other possible mechanisms may operate and should consider the need for evaluation of male fertility.

Therefore, this study was conducted to investigate the effects of long-term ingestion of fluoxetine on fertility in adult male rats.

## MATERIAL AND METHOD

*Animals and treatment*

This study was conducted with the approval of animal care committee. Adult male and female albino rats of Sprague Dawley strain, weighing about 300 g were raised in the Animal House Unit at Jordan University of Science and Technology under controlled temperature of 21±1 °C and 12:12 hr light/dark cycles. Food and water were available *ad libitum*. The control group (n=10) received vehicle. The treated group (n=10) received powdered of fluoxetine (200 mg/kg) dissolved in distal water and given by intragaseric feeding needle (gavages tube) for 60 days. The drug and vehicle were administered at 9 AM daily and were blinded for the treatment group. The half life of flouxetin was reported to be 452 mg/kg. After drug treatment for 60 days (after 24 hours of the last dose), animals were weighed and autopsied under light ether anesthesia. Blood was collected through cardiac puncture using a dry and clean syringe for serum studies.

*Fertility Test*

Fertility was estimated in adult male rats treated with fluoxetine and in control male counterparts. Each male rat was placed in an individual cage with two virgin untreated females of the same strain; they were left together for ten days, during which two estrous cycles should have elapsed (Rugh, 1968). One week after the removal of the exposed males, females were killed by cervical dislocation under light ether anesthesia and the number of pregnant females; number of implantation sites, number of viable fetuses and number of resorptions were recorded.

*Sperm Motility and Count*

To determine sperm motility and sperm counts, 100 mg of cauda epididymides was minced in 2 ml of physiological saline. One drop of an evenly mixed sample was applied to a Neubauer's counting chamber under a cover slip. Quantitative motility expressed as a percentage was determined by counting both motile and immotile spermatozoa per unit area. Cauda epididymal and testicular sperm counts were made by routine procedure and expressed as million/ml of suspension (Prasad *et al.*, 1972).

*Body and Organ Weights*

Initial and final body weights of the animals were recorded. The reproductive tract was taken out trimmed free of fat and each organ was weighed separately on an electronic balance. The male reproductive organs used for the study-included testes, epididymides, ventral prostrate, seminal vesicle and vas deferens. Vital organs such as liver, kidney, adrenal, heart and thyroid were also taken out and weighed. Reproductive organs along with a small piece of liver, heart and kidney were fixed in Bouin's fixative for histological studies.

*Histological Studies*

The Bouin's fixed reproductive organs (testes, epididymides, seminal vesicle, ventral prostate and vas deferens) along with liver, kidney and heart muscles were cut into small pieces and processed. The paraffin embedding was followed by section cutting (5 µm) and staining (Harris haematoxyline and eosin).

*Histometry*

With the help of Camera Lucida hundred circular appearing seminiferous tubules were traced at x80 and the diameter of each tubule was measured separately. The measurement was expressed as the mean of all the traced tubules. Similarly, Leydig cell nuclei were traced at x800. The epithelial cell height of cauda epididymides, caput epididymides and seminal vesicle were also traced at 360×.

*Testicular Cell Population Counting*

Spermatogenic elements i.e. spermatogonia, spermatocytes and spermatids were counted in 5 µm thick cross sections of 10 seminiferous tubules in 10 animals of each group. All raw counts were transformed to true counts by an adaptation of Abercrombie formula (Abercrombie, 1946) from the germ cell diameter measurement.

Interstitial cell types such as fibroblast, immature and mature Leydig cells and degenerating cells were estimated, applying a differential count of over 200-cell population and statistically verified by the binomial distribution (Dixon and Massey, 1957).

*Serum Biochemistry*

Total protein, cholesterol, triglycerides, serum aspartate aminotranferase (AST), and serum alanine aminotranferase (ALT) were obtained using commercial kits.

*Hormonal Assays*

Plasma FSH and testosterone concentrations were measured by Radioimmunoassay using commercial kits.

*Statistical Calculation*

Data was expressed as mean ± standard deviation (SD). The differences between fluoxetine exposed groups and control were analyzed using either Chi-square test or Student "t" test. p-values less than 0.05 were considered significant (Ipstein and Poly, 1970).

## RESULTS

*Effect of fluoxetine on body and organs weight*

Table 1 shows that administration of fluoxetine caused a slight decrease in body weight ($p<0.05$) when initial and final body weights were compared in the experimental group. On the contrary, an increase in the body weight was observed in the control group. However, the weight of the testes, epididymides, seminal vesicle, ventral prostate decreased significantly as ($p<0.001$), and was ($p<0.05$) for the vas deferens.

*Effect of fluoxetine on Sperm Dynamics and Histometrical Parameters.*

While sperm motility in cauda epididymis was significantly ($p<0.001$) decreased in treated animals in comparison to the control, sperm density, seminiferous tubule diameter and Leydig cell nuclear diameter in treated male rats were significantly ($p<0.001$) decreased. Epithelial cell height in epididymides (cauda, caput and seminal vesicle) were also considerably decreased (Table 2).

*Effect of Fluoxetine on Testicular Cell Population Dynamics.*

Table 3 demonstrates that the administration of fluoxetine caused a considerable decrease in the germinal cell population: spermatogonia, spermatocytes (primary and secondary) and spetmatids were also decreased to

**Table 1.** Effect of Fluoxetine (200 mg/kg ) on body and organ weights male rats.

| Treatment | Body weight [g] | | Testes | Epididymides | Seminal vesicle | Ventral Prostate | Vas deferens |
|---|---|---|---|---|---|---|---|
| | Initial | Final | (mg/100 g body weight) | | | | |
| Control | 267±12.21 | 296±11.76 | 925±9.83 | 395±8.62 | 404.58±5.5 | 215±3.01 | 87±1.78 |
| Fluoxetine | 275±8.35 | 247±7.66 * | 869±8.66 *** | 312±3.81 *** | 361±9.37 *** | 169±5.38 *** | 64±0.66 * |

Results are expressed as mean ± S.D.; Ten rats were included per group.
*p<0.05, **p<0.01, ***p<0.001 - significantly different from control group (Student's "t" test).

**Table 2.** Effect of Fluoxetine (200 mg/kg ) on histometerical parameters and sperm dynamics in male rats.

| Treatment | Sperm motility % | Sperm density million/ml | | Seminiferous tubule | Leydig cell nuclear | Epithelial cell height | | |
|---|---|---|---|---|---|---|---|---|
| | Cauda | Testes | Cauda | diameter | diameter | Caput | Cauda | Seminal vesicle |
| | | | | µm | | | | |
| Control | 74.1±1.94 | 4.75±0.47 | 56.0±1.94 | 290.6±3.2 | 6.45±0.96 | 38.8±0.4 | 26.08±0.32 | 17.32±0.17 |
| Fluoxetine | 9.23±.0.33 *** | 1.12±0.65 *** | 4.4±1.66 *** | 65.3±2.8 *** | 3.07±0.9 *** | 10.47±1.69 *** | 11.23±1.77 *** | 7.96±1.08 *** |

Results are expressed as mean ± S.D.; Ten rats were included per group.
*p<0.05, **p<0.01 ***p<0.001 - significantly different from control group (Student's "t" test).

**Table 3.** Effect of Fluoxetine (200 mg/kg) on testicular cell population dynamics in male rats.

| Treatment | GERMINAL CELL TYPES | | | | | INTERSTITIAL CELL TYPE | | |
|---|---|---|---|---|---|---|---|---|
| | Spermatogonia | Spermatocyte (primary) | Spermatocyte (secondary) | Spermatids | Fibroblast | Immature Leydig cell | Mature Leydig cell | Degenerating cell |
| Control | 23.99±0.93 | 18.85±0.80 | 64.126±3.51 | 147.71±4.87 | 63.83±1.64 | 65.20±3.47 | 70.64±1.03 | 18.34±1.67 |
| Fluoxetine | 13.54±1.69 *** | 9.25±1.66 *** | 12.35±1.96 *** | 42.36±1.4 *** | 34.32±1.8 *** | 36.75±2.11 *** | 38.18±1.66 *** | 53.16±1.49 *** |

Results are expressed as mean ± S.D., Ten rats were included per group.
***p<0.001 - significantly different from control group (Student's "t" test).

**Table 4.** Effect of Fluoxetine (200 mg/kg) on serum biochemistry in male rats.

| Treatment | Glucose Mmol | Cholesterol Mmol | Triglycerides Mmol | Bilirubin µmol | ALT U/L | AST U/L | Testosteron nmol/l | FSH Iu/L |
|---|---|---|---|---|---|---|---|---|
| Control | 7.3±0.212 | 1.4±0.147 | 0.8±0.07 | 3.175±0.142 | 36.7±1.66 | 77.7±2.12 | 14.4±2.53 | 21.87±0.47 |
| Fluoxetine | 6.07±0.11 * | 1.18±0.07 * | 0.7±0.33 * | 2.67±0.47 * | 104±2.33 *** | 121±1.75 *** | 6.75±2.04 *** | 16.17±0.45 *** |

Results are expressed as mean ± S.D., Ten rats were included per group.
*p<0.05, ***p<0.001 - significantly different from control group (Student's "t" test).

**Table 5.** Effect of Fluoxetine (200 mg/kg) on rats fertility in male rats.

| Treatment | No.of male | No.of female | No.of pregnant females | No.of implantation sites | No.of viable fetuses | No. of resorption /total No. of implantation sites |
|---|---|---|---|---|---|---|
| Control | 10 | 20 | 18/20 (90%) | 9.63±2.66 | 9.37±1.16 | 4/173(2.31%) |
| Fluoxetine | 10 | 20 | 8/20† (40%) | 5.36***±2.83 | 5.25 ***±1.67 | 9/43† (21%) |

Results are expressed as mean ± S.D., Ten rats were included per group.
***p<0.001 - significantly different from control group (Student's "t" test). † p<0.05 (chi -square test).

a significant (p<0.001) level. Similarly the Fibroblast, immature and mature Leydig cells numbers were also considerably decreased. However, the degenerating cells number was greatly increased (p<0.001).

*Effect of Fluoxetine on Serum Biochemical Markers*

Results presented in Table 4 show that glucose, bilirubin, total cholesterol and triglycerides levels were within the normal range. Serum level of AST and ALT results were found to be significantly increased in the treated group when compared to the control. On the contrary, levels of plasma testosterone and FSH were significantly decreased (p<0.001) in the treated group when compared to the control group.

*Effect of fluoxetine on male rat fertility*

Table 5 demonstrates a significant decrease in the number of females impregnated by fluoxetine treated male rats. The number of implantations and number of viable fetuses were also considerably decreased in female rats impregnated by those male rats ingested fluoxetine. On the other hand, the number of resorptions was significantly increased in females impregnated by male rats ingested fluoxetine.

Fluoxetine significantly increased the post ejaculatory interval and its ingestion significantly reduced the number of ejaculating males.

## DISCUSSION

In this study the effects of long-term exposure of an adult male rat to 200 mg/kg concentration of fluoxetine, on fertility and reproductive system were investigated.

Up to date, there is a shortage of data on the effects of long-term ingestion of fluoxetine on various parameters of biological behaviors and reproductive capacity in adult male rats. These facts prompted the authors of this article to initiate this study. The animal model used in this work

has been previously used to assess the adverse effects of metal salts ingestion on behavior and fertility in small laboratory animals (Bataineh *et al.*, 1998) without compromising the health of the experimental animals.

The dose of 200 mg/kg of powdered fluoxetine (200 mg/kg) dissolve in distal water and given by intragaseric feeding needle (gavages tube) for 60 days was selected because of the reported toxicity potentials of higher doses of this compound including decreased body weight and water consumption and clinical signs of toxicity such as dehydration, lethargy and hunched posture (Fail *et al.*, 1991). This dose level was also selected to obtain broader range of information on the effects of fluoxetine on behavior parameters and reproduction.

In rats, the whole spermatogenic process requires 53 days out of which spermatozoa spend the last 6 to 7 days in the final transit through epididymides (Ke and Tso, 1982). Fluoxetine was administrated for one complete spermatogenic cycle.

The present investigation shows that oral administration of fluoxetine promoted decreased fertility in male albino rats. The weight of reproductive organs were markedly decreased (Table 1). The weight, size and secretory functions of testes, epididymes, and seminal vesicles, ventral, prostate, vasa and deferentia are closely regulated by androgens (Choudhary and Steinberger, 1975; Agrawal *et al.*, 1986). The drug may act on pituitary gland and increase the main hormone of spermatogenesis. The process of spermatogenesis and accessory reproductive organs function are androgen dependent. Increased androgen production is reflecting a decrease in the number of mature Leydig cells and their functional status. In the present study the number of degenerating Leydig cells were significantly decreased, this reflects the decrease of androgen level. It is further confirmed by decreased number of spermatocytes (both primary and secondary) and spermatids as these stages are completely androgen dependent (Dym *et al.*, 1979). The decrease in weight and histometry of reproductive organs further confirmed androgen increase. Significant decrease in the sperm motility of cauda epididymis was observed in the treated group. This may be due to the effects of fluoxetine on the enzymes of oxidative phosphorylation.

The results presented in this paper show that ingestion of fluoxetine by adult male rats decrease the number of females impregnated by the exposed males (Table 5). Also, the number of implantations and the number of viable fetuses were decreased. This decrease appearing to this effect may be due to decrease in sperm motility and sperm density.

In conclusion, these results confirm that the long-term fluoxetine ingestion produce adverse effects on fertility and reproductive system in adult male rat possibly through generalized toxicity and explain the reduction in the levels of glucose, cholesterol, triglycerides and bilirubin. Thus, it would be of great interest to investigate the impact via long –term treatment with fluoxetine in male human fertility.

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