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Original Article

Examining the Anatomical Relationship: Serum GnRH, Vitamin E and Ovarian Follicular Atresia in Fluoxetine-Treated Rats

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Abstract

Objective: To determine whether fluoxetine-induced suppression of serum gonadotropin-releasing hormone (GnRH) is matched by increased ovarian follicular atresia, and whether vitamin E alters either.

Methodology: Sixty adult female Wistar albino rats aged 8–12 weeks, each with three consecutive regular estrous cycles, were randomized to six groups of ten. Groups A and F received distilled water and olive oil (vehicle controls); B and C received oral fluoxetine at 20 and 80 mg/kg/day, respectively; and D and E received the same doses plus vitamin E at 250 mg/kg/day, for 15 days. We used ELISA to assay serum GnRH; we stained ovaries with hematoxylin and eosin and counted atretic follicles with a calibrated ocular micrometer. Analysis used ANOVA with post hoc Tukey and Fisher exact tests.

Results: Mean serum GnRH was 36.54±1.56, 20.86±1.92, 15.22±2.13, 35.88±0.98, 35.49±1.42 and 36.45±1.97 pg/ml in Groups A to F ($p = 0.0001$). Atresia was raised in 19 of 60 rats (31.7%): 8 in Group B, 10 in Group C, 3 in Group E, none in controls ($p = 0.0001$).

Conclusion: Fluoxetine lowered GnRH and increased follicular atresia, dose for dose. Vitamin E prevented nearly all of it.

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Introduction

Between 11% and 20% of women of reproductive age take an antidepressant[1]. Depression is the second most common chronic condition in this group, and selective serotonin reuptake inhibitors (SSRIs) are what most of them are given first[2]. Fluoxetine is among the most widely dispensed. These drugs are built to act on the brain, but they do not stop there, and SSRI exposure is now known to disturb several endocrine axes, the gonadal axis included[3]. Reviews of the reproductive literature report altered steroid output, disordered folliculogenesis, and reduced fertility in both sexes. The clinical signal is not trivial either. Women treated for a first depressive episode showed significant falls in anti-Müllerian hormone and antral follicle count after six months of SSRI therapy, which puts the ovarian reserve itself in question[4]. How a serotonergic drug reaches the ovary is reasonably well mapped. GnRH is released in pulses, and that pulsatility is the signal the whole axis depends on; researchers have worked out the oscillator behind it in detail over the past decade[5]. Arcuate kisspeptin neurons co-expressing neurokinin B and dynorphin are now accepted as its core[6]. Their synchronized firing sets GnRH pulse frequency and, through that, pituitary output of follicle-stimulating and luteinizing hormones[7]. Serotonergic fibers run onto this network. Raising synaptic serotonin should therefore change GnRH release, and in rodents, fluoxetine interrupts estrous cyclicity and shifts uterine estrogen signaling[8,9]. The ovary is not only a receiver. Serotonin transporters and receptors sit inside the follicle itself, and fluoxetine blocks serotonin uptake by the oocyte while downregulating growth differentiation factor 9, degrading the dialogue between oocyte and granulosa cells[10].In mice, chronic exposure cuts the number of ovulated oocytes and slows cytoplasmic maturation even when gonadotropins look normal. Vitamin E (α-tocopherol) is the main lipid-soluble antioxidant of cell membranes, and its protective role in female reproductive tissue, limiting lipid peroxidation and holding membranes together, is established[11]. Antioxidants have accordingly been proposed as adjuncts in infertility, though neither agent nor dose has been settled. This study therefore measured serum GnRH and counted atretic follicles in the same rats after 15 days of oral fluoxetine at two doses, given with and without vitamin E, so the hormonal and structural responses could be compared across a dose range.

Materials and Methods

This study was conducted in the Department of Anatomy, Allama Iqbal Medical College, Lahore, from January 2026 to June 2026, over six months after receiving institutional review board approval. Wistar albino rats (healthy adult females aged 8-12 weeks) were obtained from the institutional animal house. Standard laboratory conditions of 23 ± 2 °C, 55 ± 5% relative humidity, and a 12-hour light/dark cycle were maintained, and animals were fed standard chow and water ad libitum. After acclimatization, only rats with three normal estrous cycles in one week were used. Vaginal smears were used to evaluate estrous stages using a light microscope [11]. Rats were randomly divided into six groups (A–F) of 10 animals each. We administered fluoxetine hydrochloride at 20 and 80 mg/kg/day and vitamin E at 250 mg/kg/day. Vehicle controls (distilled water and olive oil) were kept. All the treatments were given orally by gavage once a day for 15 days, as shown in Table 1. We collected blood samples by cardiac puncture from anesthetized animals at the end of the treatment. We separated the serum by centrifugation and stored it at −80 °C for further analysis. We measured serum gonadotropin-releasing hormone (GnRH) levels in duplicate using a species-specific enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. We removed the ovaries, macroscopically examined them, and fixed them in 10% buffered formalin for 48 hours. After routine tissue processing, we stained 5-µm sections with hematoxylin and eosin. We examined the four slides from each animal under a microscope. We identified atretic follicles by granulosa cell degeneration, nuclear pyknosis, cellular debris, and degeneration of the oocyte and zona pellucida. A blinded reviewer evaluated follicular changes and graded them as normal and/or increased compared with the control groups. We used SPSS version 21.0 to analyze the data. We presented GnRH levels as means ± SD and compared them using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. We reported atretic follicle counts as frequencies and percentages and compared them using Fisher's exact test. We considered $P \leq 0.05$ statistically significant.

Results

All 60 rats completed the 15 days and were analyzed. None died, and none showed overt systemic toxicity. Final body weights were close to baseline in every group, and screening before allocation confirmed three successive regular cycles in each animal. At dissection, the ovaries looked unremarkable to the naked eye, with no gross distortion, adhesion, or cystic change anywhere in the series; what followed was apparent only on assay and under the microscope. Serum GnRH on day 15 differed across the six groups ($p=0.0001$). Figure 1 shows how the group means fell out. Both fluoxetine doses sat well below the two vehicle controls, while the groups given vitamin E alongside fluoxetine stayed inside the control range. The gap

between Group B and Group C matters, because it shows the effect tracking dose rather than simply switching on.

Table 1: Animal Groups and Experimental Intervention Design.

Group	Intervention	Dose and vehicle	Duration	Route
A	Vehicle control (water)	1 mL distilled water	15 days	Oral
B	Fluoxetine, low dose	20 mg/kg/day in 1 mL distilled water	15 days	Oral
C	Fluoxetine, high dose	80 mg/kg/day in 1 mL distilled water	15 days	Oral
D	Fluoxetine (low) + vitamin E	20 mg/kg/day + 250 mg/kg/day in 1 mL olive oil	15 days	Oral
E	Fluoxetine (high) + vitamin E	80 mg/kg/day + 250 mg/kg/day in 1 mL olive oil	15 days	Oral
F	Vehicle control (olive oil)	1 mL olive oil	15 days	Oral

Ten female rats were allocated to each group (n = 10). Groups A and F served as vehicle controls for distilled water and olive oil, respectively. All interventions were administered orally for 15 consecutive days.

Figure 1. **Mean Serum GnRH Levels in Female Rats Following 15 Days of Experimental Treatment**

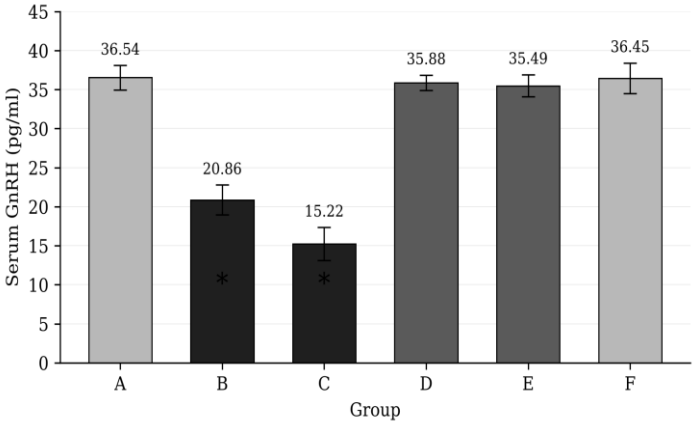


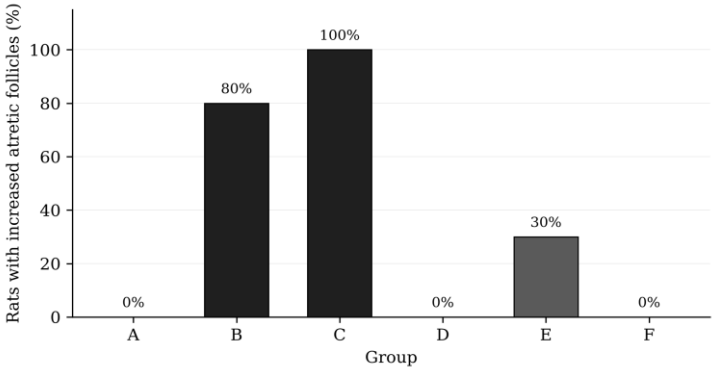
Figure 1: Mean serum GnRH (pg/ml) of female rats on day 15 after 15 days of oral fluoxetine (20 or 80 mg/kg/day; Groups B and C) and vitamin E (250 mg/kg/day; Groups D and E). Bars represent the mean ± standard deviation of 10 rats per group. Groups A and F are the water and olive oil controls. One-way ANOVA, $p = 0.0001$. *$p \leq 0.05$ versus Group A.

Table 2: Multiple Comparisons of Mean Serum GnRH Levels Among Experimental Groups Using Tukey's Post Hoc Test

Comparison	I Group	J Group	Mean Difference (I–J)	p-value
Control vs. low-dose fluoxetine	A	B	15.67*	0.000*
Control vs. high-dose fluoxetine	A	C	21.31*	0.000*
Control vs. low-dose fluoxetine + vitamin E	A	D	0.66	0.953
Control vs. high-dose fluoxetine + vitamin E	A	E	1.05	0.743
Water vs. olive oil control	A	F	0.08	1.000
Low-dose vs. high-dose fluoxetine	B	C	5.64*	0.000*
Low-dose fluoxetine vs. rescue group	B	D	−15.01*	0.000*
High-dose fluoxetine vs. rescue group	C	E	−20.26*	0.000*
Rescue groups comparison	D	E	0.38	0.996

$p \leq 0.05$ is statistically significant. We performed pairwise comparisons of mean serum GnRH levels (pg/mL) using Tukey's post hoc test following one-way ANOVA. Mean differences are expressed as Group I minus Group J. An asterisk (*) indicates a statistically significant difference at $p \leq 0.05$.

Figure 2: Proportion of Female Rats Showing Increased Ovarian Atretic Follicle Counts in Each Experimental Group.



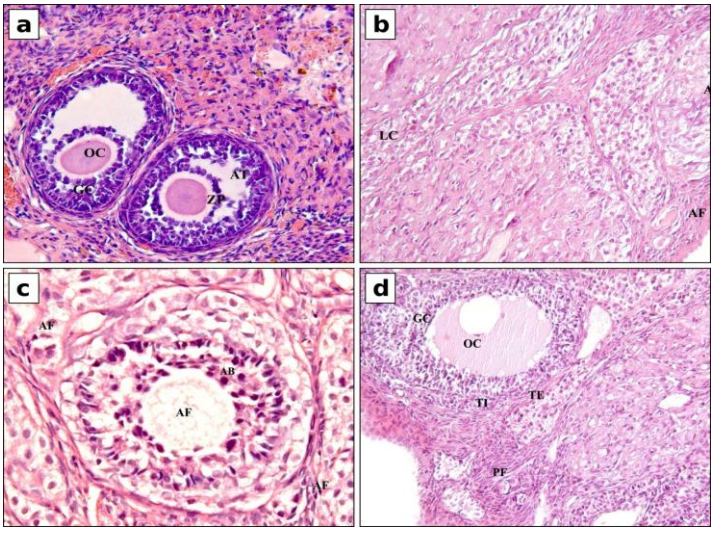
Bars represent the percentage of female rats with increased atretic follicle counts in each experimental group after 15 days of treatment. Each group consisted of 10 rats (n = 10). We assessed group differences using Fisher's exact test (p = 0.0001).

Table 3: Effect of Fluoxetine and Vitamin E on Ovarian Atretic Follicle Counts in Female Rats After 15 Days of Treatment

No. of atretic follicles	Group A	Group B	Group C	Group D	Group E	Group F	Total
Normal	10 (100.0%)	2 (20.0%)	0 (0.0%)	10 (100.0%)	7 (70.0%)	10 (100.0%)	41 (68.3%)
Increased	0 (0.0%)	8 (80.0%)	10 (100.0%)	0 (0.0%)	3 (30.0%)	0 (0.0%)	19 (31.7%)
Total	10	10	10	10	10	10	60

Values are numbers of rats and percentages for 10 rats in each group and for the total of 60 rats. Fisher exact test = 47.03, p = 0.0001*. *p ≤ 0.05 is statistically significant.

Figure 3. Histopathological Features of Ovarian Sections in Female Rats Following Experimental Treatment



Representative photomicrographs of ovarian sections on day 15: (a) Group A (water control), showing antral follicles with oocyte (OC), zona pellucida (ZP), granulosa cell layers (GC), and antral space (AT), with no lymphocytic infiltration in the ovarian stroma; (b) Group B (fluoxetine 20 mg/kg/day), showing atretic follicles (AF), degenerating zona pellucida, and lymphocytic infiltration (LC) of the stroma; (c) Group C (fluoxetine 80 mg/kg/day), showing atretic follicles (AF), loss of granulosa cell layers, and apoptotic bodies (AB); and (d) Group D (fluoxetine 20 mg/kg/day plus vitamin E 250 mg/kg/day), showing a primordial follicle (PF) and an antral follicle with oocyte (OC), granulosa cells (GC), theca interna (TI), and theca externa (TE), with no stromal infiltration—Hematoxylin and eosin staining; original magnification ×200.

Discussion

Fluoxetine lowered serum GnRH and raised follicular atresia in the same animals, in the same dose order, and vitamin E reversed both. When a hormone measurement and a morphological one move together across a dose range, the simplest reading is that one is driving the other. Rats given sofosbuvir show something comparable: the drug raised atretic follicle counts. It cut follicular and luteal numbers, and vitamin E given alongside restored oxidative stress markers and histology.[12-16] Stereological work in hyperthyroid rats points in the same direction, since vitamin E preserved antral follicle and corpus luteum volumes along with serum follicle-stimulating hormone, suggesting it protects the pituitary–ovarian axis as a whole rather than the gonad alone.[17] The mechanism that fits is withdrawal of gonadotropic support, pushing antral follicles into apoptosis. Psychotropic drugs are known to unsettle this axis. Haloperidol in female rats shifted circulating gonadotropins and anti-Müllerian hormone while raising ovarian oxidative, inflammatory, and apoptotic markers, and an antioxidant normalized the hormonal and histological picture together.[18] A purely hormonal account, however, will not stretch to cover Group E. Vitamin E restored GnRH control at both doses, yet three rats in ten at the higher dose still had excess atresia. Fluoxetine is itself a source of reproductive oxidative injury: in Wistar rats it depleted catalase and glutathione peroxidase and damaged gonadal histoarchitecture, and an exogenous antioxidant undid both.[19] So the second route is probably oxidative and local. Granulosa cells sustain high rates of steroidogenesis and

mitochondrial respiration and therefore work close to the limit of their antioxidant reserve, which is why mitochondrial trouble converts so readily into granulosa cell death and follicular loss.[20] Oxidative stress drives these cells into senescence and contributes to premature ovarian insufficiency both in culture and in vivo.[21] The reverse also holds: restoring redox balance through the NRF2/HO-1 pathway rescues defective follicular development in injured ovaries, marking the follicle wall as a target an antioxidant can reach.[22] Read this way, 250 mg/kg/day of vitamin E was enough to cover the hypothalamic and pituitary end of the axis at both doses and the follicle itself at the lower dose, but was outrun at 80 mg/kg/day in a minority of animals. A single GnRH sample, a binary atresia score, and the absence of gonadotropin and ovarian redox measurements leave the intermediate steps inferred rather than shown, and the higher dose sits above the human therapeutic range.

Conclusion

Fifteen days of oral fluoxetine suppressed serum GnRH in adult female albino rats in a dose-related way and produced a matching, dose-related rise in ovarian follicular atresia, with fewer and smaller corpora lutea and lymphocytes in the stroma. Because the hormonal and structural findings align across the dose range, the atresia appears largely downstream of reduced gonadotropic support. Vitamin E at 250 mg/kg/day restored GnRH at both doses and fully preserved the follicular population at the lower dose, but at the higher dose left a minority of ovaries with excess atresia despite normal hormone levels, suggesting a second, likely oxidative, insult acting on the follicle itself. Measuring gonadotropins and ovarian oxidative markers alongside these endpoints would separate the two pathways directly.

Ethical Approval

The Hospital Research and Ethics Committee (IREB) approved the study under Reference No. 341/AIMC/06/2023 before the study began.

Informed Consent

We obtained written informed consent from all participants before enrolment. We informed participants and their parents or legal guardians about the study objectives, procedures, and the voluntary nature of participation. Participation was entirely voluntary.

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This study received no external funding and used available institutional resources.

Conflict of Interest

The authors declare no competing interests or conflicts of interest related to this study.

Authors' Contributions

Syeda Rizwana Jafri: Contributed to conceptualization, study design, data collection, interpretation of findings, manuscript drafting, critical revision, and final approval. He also accepts accountability for all aspects of the work.

Asma Fayyaz, Arshia Mobeen Rana, Afifa Arshad: Contributed to study design, data collection, data curation, literature review, data interpretation, manuscript revision, and final approval. They also accept accountability for all aspects of the work.

Hira Sikandar, Zunaira Kanwal: Contributed to conceptualization, study supervision, clinical assessment, interpretation of findings, and critical revision of the manuscript for important intellectual content. She also approved the final version and accepts accountability for all aspects of the work.

All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work in accordance with the ICMJE authorship criteria.

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