Aceh Journal of Animal Science (2025) 10 (3): 154-159

Aceh Journal of Animal Science

Journal homepage: www.jurnal.usk.ac.id/AJAS

Impact of Antidepressant Fluoxetine on Female Fertility Using Zebrafish (*Danio rerio*) as an Experimental Model

Teja Mothe[1], Mehdi A. Mirza[1*], S Seethalakshmi[1]

[1] *Department of Pharmacology, ESIC Medical College and Hospital, Sanathnagar, Hyderabad, Telangana, India-500038*

ARTICLE INFO

Keywords:
Infertility
Toxicity
Micro-pollutants
Contamination

Received: 29 May 2025
Accepted: 03 November 2025

DOI:10.13170/ajas.10.3.46473

ABSTRACT

Aquatic ecosystems are increasingly contaminated by pharmaceutical pollutants, including antidepressants such as fluoxetine, a selective serotonin reuptake inhibitor (SSRI). Detected in water-bodies, wastewater, and fish, fluoxetine poses potential risks to environmental and reproductive health. This study investigates the impact of fluoxetine exposure on zebrafish fertility, as SSRIs are known to influence reproductive function in humans. Five adult male-female zebrafish pairs were maintained in 10-litre tanks at 28°C under a 14-hour light and 10-hour dark cycle and fed a high-protein diet thrice daily. To establish baseline fertility, each pair was initially spawned once, and the resulting eggs were discarded. The experiment was conducted over three cycles. In Cycle-1, pairs were separated for five days with daily water changes and allowed to spawn on Day-6. Total eggs and live eggs (clear and transparent) were recorded, with dead eggs (cloudy and opaque). In Cycle-2, both sexes were exposed to 3.2 µg/L fluoxetine daily for five days before spawning. In Cycle-3, the same protocol as Cycle-1 was repeated without fluoxetine to observe delayed effects. Mean egg-counts were 231(±14) in Cycle-1, 427(±57) in Cycle-2, and 442(±31) in Cycle-3. Significant difference ($p<0.05$) between Cycle-1 vs Cycle-2 and Cycle-1 vs Cycle-3 was observed, while no significant difference was found between Cycle-2 and Cycle-3 ($p>0.05$). Similarly, the proportion of live eggs declined across cycles: 47%(543/1155) in Cycle-1, 33%(704/2139) in Cycle-2, and 29%(641/2212) in Cycle-3. The decline was statistically significant between Cycle-1 vs Cycle-2 and Cycle-1 vs Cycle-3 ($p<0.05$), but the difference between Cycle-2 and Cycle-3 was not significant ($p>0.05$). A strong negative correlation was observed between total egg output and the proportion of live eggs. These findings suggest that fluoxetine enhances egg production while reducing egg viability. This underscores the reproductive toxicity of environmental fluoxetine exposure in aquatic species, with potential implications for human health. The study highlights pharmaceutical pollution's interconnected ecological and reproductive impacts.

Introduction

Fluoxetine (FLX) is a widely used selective serotonin reuptake inhibitor (SSRI) increasingly detected as a micropollutant in aquatic environments. The SSRIs persist through wastewater treatment and accumulate in fish, raising concerns that they act as endocrine-disrupting compounds (EDCs) in wildlife (Dorelle *et al.,* 2017). Serotonin (5-HT) intimately regulates vertebrate reproductive hormones (e.g. GnRH, LH, estrogen), so chronic FLX exposure can perturb the hypothalamo-pituitary-gonadal (HPG) axis (Thompson *et al.,* 2022). This study attempts to elucidate the reproductive toxicity potential of fluoxetine at environmentally and pharmacologically relevant concentrations. In humans, SSRIs, including fluoxetine, are known to cause sexual dysfunction (reduced libido, delayed orgasm) in some women. A few small clinical reports suggest SSRI use can raise serum prolactin and impair ovulation (Jing *et al.,* 2016; Reeves *et al.,* 2016).

Rodent models likewise show that chronic FLX disrupts female fertility. In Fischer rats given high-dose FLX (10 mg/kg/day), the estrous cycle was significantly lengthened or abolished and serum progesterone plummeted. These females lost sexual

* Corresponding author.
Email address: mehdialimirza@gmail.com

Printed ISSN 2502-9568; Electronic ISSN 2622-8734

receptivity (lordosis) until compensatory neuroendocrine adaptations restored cycling after ~3 weeks (Maswood *et al.,* 2008).

Zebrafish (*Danio rerio*) are a premier model for reproductive toxicology due to their well-characterized ovarian cycle, genetic similarity to humans, transparent embryonic development, high fecundity and ease of maintenance. Zebrafish ovaries share homologous features with human ovaries in terms of oocyte maturation and hormonal regulation, making them an ideal system to investigate drug-induced reproductive effects (Zha *et al.,* 2024). Moreover, their aquatic environment allows for controlled drug exposure via waterborne routes, mimicking environmental contamination and systemic drug exposure patterns (Cassar *et al.,* 2020). Zebrafish reproduce by daily spawning once sexually mature, with oocyte growth regulated by pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In female zebrafish, FSH signaling drives follicular growth, whereas LH triggers oocyte maturation and ovulation (Li *et al.,* 2020). Female fertility-related endpoints in zebrafish include behavioral parameters, physiological measures (such as hormone levels and ovary morphology), reproductive output (e.g., egg production and spawning), and molecular changes (including gene expression and endocrine markers) (Hoo *et al.,* 2016).

This research aims to evaluate and confirm the effects of fluoxetine exposure on female fertility in zebrafish under controlled laboratory conditions. The concept of assessing fluoxetine's environmental toxicity via fish is already well-supported and relevant in natural aquatic environments (Dorelle *et al.,* 2017). The study will specifically assess parameters such as egg-laying capacity and egg viability in female zebrafish exposed exclusively to fluoxetine. The outcomes of this research could provide valuable insights into the reproductive risks of SSRI exposure in aquatic ecosystems and contribute to the broader understanding of drug-induced fertility alterations in women of childbearing age.

Materials and Methods

Study Setting and Design

This prospective, non-clinical, interventional study was conducted over a period of six months (June to November 2024) in the Zebrafish Laboratory, Department of Pharmacology, ESIC Medical College, Sanathnagar, Hyderabad. The study was approved by the Institutional Animal Ethics Committee (1864/PO/Re/S/16/CPCSEA0103-2024). The study aimed to assess the effect of fluoxetine exposure on zebrafish fertility parameters including egg production and viability.

Study Population

The study involved adult male and female wild-type zebrafish (*Danio rerio*), procured from a standardized aquatic supplier and acclimatized in laboratory conditions for two weeks prior to experimentation. Healthy fish displaying normal swimming behavior and physical appearance were selected for the study. A total of five breeding pairs (n = 10 zebrafish) were used.

Housing and Husbandry Conditions

Each breeding pair was maintained in a separate 10-litre glass tank containing dechlorinated water maintained at a constant temperature of 28°C. The water was changed daily to maintain optimal water quality. A controlled photoperiod was maintained using a programmable digital timer set to a 14-hour light and 10-hour dark cycle to simulate natural environmental conditions and promote reproductive behavior. The zebrafish were fed a high-protein diet three times daily throughout the study period.

Materials Used

The materials used in this study included commercially available Fluoxetine Capsule 20 mg (test drug), standard zebrafish housing and experimental equipment. Fish were maintained in individual 10-litre capacity glass tanks and spawning was facilitated using custom-designed spawning chambers. A programmable digital timer was employed to maintain a controlled 14-hour light and 10-hour dark cycle. The zebrafish were fed a high-protein diet formulated specifically for optimal reproductive performance. Egg observation and viability assessment were carried out using an entomology microscope with 20X magnification. For accurate measurement of drug quantities and fish feed, an analytical balance was used. Eggs were collected in sterile petri dishes and manually counted using a PetriSticker square grid adhered to the base of each dish to ensure consistent visualization and quantification.

Experimental Procedure

The study was conducted in four sequential phases to evaluate the effects of fluoxetine on zebrafish fertility: Baseline Fertility Screening, Cycle-1 (Pre-treatment), Cycle-2 (Treatment), and Cycle-3 (Post-treatment). In the Baseline Fertility Screening phase, each adult male-female zebrafish pair was separated for five consecutive days, with daily water changes to simulate natural conditions and promote gamete maturation. On the sixth day, the pairs were introduced into spawning chambers to allow natural mating. The eggs produced during this phase were

intentionally discarded to eliminate the influence of previous reproductive cycles and to ensure that all pairs had achieved reproductive readiness for the subsequent experimental cycles.

Cycle-1

Pre-treatment Phase commenced. The fish were again separated by sex for five days, with daily water changes maintained. On Day 6, the pairs were reunited in spawning chambers and allowed to mate. The total number of eggs laid was counted immediately after spawning. To determine egg viability, the eggs were transferred into petri dishes labeled with PetriSticker square grids and examined 12 hours post-fertilization using a 20X magnification entomology microscope. Live eggs were identified as clear and transparent, while dead eggs appeared cloudy and opaque.

Cycle-2

Treatment Phase, both male and female zebrafish were separated and individually exposed to fluoxetine for five days. The fluoxetine was administered at a concentration of 3.2 µg/L, which was added daily after the water change. This dose was chosen based on literature as it represents an environmentally relevant concentration (Weinberger *et al.*, 2014). On the sixth day, the fish were transferred to the spawning chambers for mating. The total number of eggs and the number of live eggs were recorded using the same protocols as in the previous cycle, to assess the acute impact of fluoxetine exposure on reproductive outcomes.

Cycle-3

Post-treatment Phase was conducted to evaluate any delayed or residual effects of fluoxetine. Without any further drug exposure, the fish were again separated by sex for five days with continued water changes. On the sixth day, the fish were allowed to spawn, and the total number of eggs and the number of live eggs were documented using the same methodologies as earlier.

The primary outcome measures assessed across all cycles included the total number of eggs laid per pair and the number and proportion of live eggs.

Statistical Analysis

All data were entered into Microsoft Excel and analyzed using GraphPad Prism version 10. Descriptive statistics were used to report the data as mean ± standard deviation (SD). Repeated Measures Analysis of Variance (ANOVA) was used to compare Cycle-1, Cycle-2 and Cycle-3 for total number of eggs and live eggs. The association between total eggs and the proportion of live eggs was assessed using Pearson's correlation coefficient. A p-value of <0.05 was considered statistically significant.

Results

All five pairs of adult male and female wild-type zebrafish (Danio rerio) were confirmed to be fertile during the baseline fertility screening and remained healthy and reproductively active throughout the study duration. No mortality or loss of reproductive behavior was observed across the experimental timeline. A total of three reproductive cycles were analyzed: Cycle-1 (Pre-treatment), Cycle-2 (Fluoxetine Treatment), and Cycle-3 (Post-treatment).

Egg Production

A statistically significant increase in the total number of eggs laid per pair was observed following fluoxetine exposure (Cycle-2) and continued into the post-treatment phase (Cycle-3), when compared to the pre-treatment phase (Cycle-1). The mean number of eggs laid in Cycle-1 was 231 ± 14, which nearly doubled in Cycle-2 to 427 ± 57, and further increased slightly in Cycle-3 to 442 ± 31. The increase in mean egg production between Cycle-1 and Cycle-2 ($p = 0.01$) as well as Cycle-1 and Cycle-3 ($p = 0.01$) were statistically significant. While between Cycle-2 and Cycle-3 ($p = 0.34$) was not significant. Results are presented in Table 1 and Figure 1.

Table 1. Total number of eggs

	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Mean ± SD
Cycle-1	233	217	242	216	248	231 ± 14
Cycle-2	348	486	440	390	470	427± 57
Cycle-3	422	430	410	470	480	442 ± 31

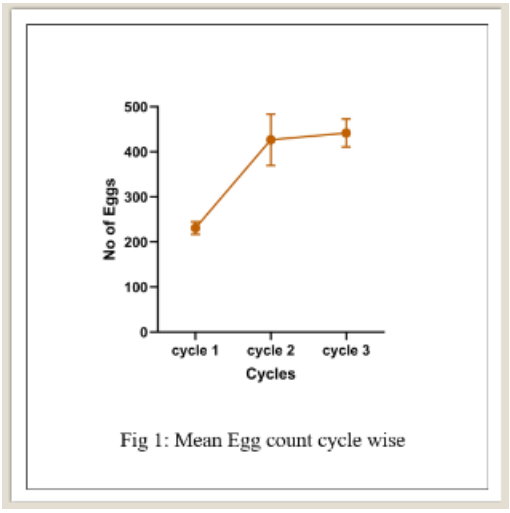

Figure 1. Mean Egg Count Cycle Wise

Egg Viability

Despite the increased egg production, the percentage of live eggs showed a consistent and significant decline across the cycles. In Cycle-1, 47% of the total 1155 eggs laid were live (n = 543 live eggs). This viability decreased in Cycle-2 to 33% of 2134 eggs (n = 704 live eggs) and further declined in Cycle-3 to 29% of 2212 eggs (n = 641 live eggs). The proportion of live eggs between Cycle-1 and Cycle-2 (p = 0.01) as well as Cycle-1 and Cycle-3 (p = 0.01) were statistically significant. While between Cycle-2 and Cycle-3 (p = 0.45) was not significant. Results are presented in Table 2 and Figure 2.

Table 2. Total number of live eggs

	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Mean ± SD
Cycle-1	105	108	110	106	117	109 ± 5
Cycle-2	122	153	145	137	149	141 ± 12
Cycle-3	119	128	124	141	139	130 ± 10

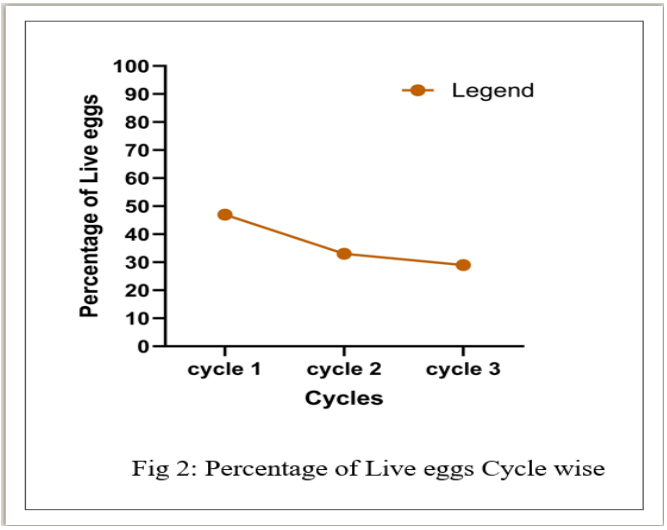

Figure 2. Percentage of Live eggs Cycle Wise

Correlation Analysis

Pearson's correlation analysis revealed a strong negative linear relationship between the total number of eggs laid and the proportion of live eggs (r = -0.96, R^2 = 0.92, p = 0.001). This inverse relationship indicates that as fecundity increased, egg viability decreased markedly, particularly in the treatment and post-treatment phases. The regression model suggests that the observed increase in egg laying induced by fluoxetine was associated with a proportionally greater decline in the quality or developmental potential of the embryos. Illustrated in Figure 3.

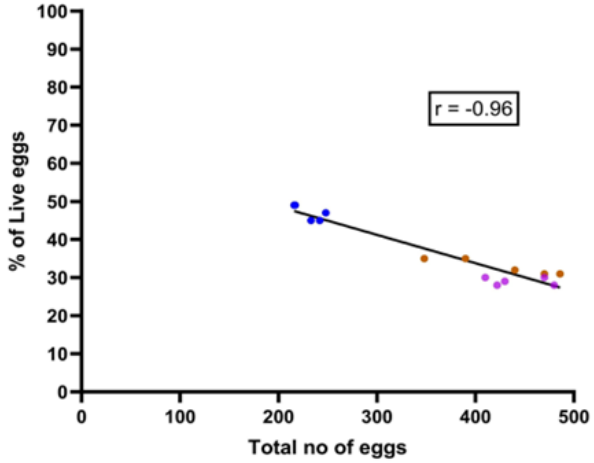

Figure 3. Correlation of total no of eggs vs % of Live eggs

Discussion

This study investigated the impact of fluoxetine exposure on female fertility using zebrafish as an experimental model, with a focus on egg production and egg viability. The findings revealed a significant increase in total egg production following fluoxetine exposure, accompanied by a substantial and progressive decline in egg viability. These results highlight a complex, dual-effect of fluoxetine on reproductive outcomes, with implications for understanding the reproductive toxicity of selective serotonin reuptake inhibitors (SSRIs).

The stimulatory effect on oviposition observed in Cycle-2 (Treatment phase) and sustained in Cycle-3 (Post-treatment phase) aligns with prior studies that suggest serotonergic modulation of the hypothalamic-pituitary-gonadal (HPG) axis (Serré *et al.,* 2021). Serotonin (5-HT), the primary target of SSRIs like fluoxetine, plays a crucial neuromodulatory role in reproductive hormone release (Ruiz-Santiago *et al.,* 2024). Fluoxetine's inhibition of serotonin reuptake increases extracellular 5-HT levels, which can activate gonadotropin-releasing hormone (GnRH) neurons and indirectly elevate levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), promoting folliculogenesis and ovulation (Perez-Caballero *et al.,* 2014). This mechanism likely underpins the observed increase in fecundity.

However, the paradoxical decline in live egg percentage across the cycles indicates a disruption in egg quality or early embryonic development. This may reflect fluoxetine-induced dysregulation at the level of oocyte maturation, fertilization, or embryogenesis. Several studies in zebrafish and other aquatic organisms have reported similar findings, where SSRIs increase spawning but impair gamete

viability, hatching success or larval survival. For instance, Brooks et al. demonstrated that chronic fluoxetine exposure in fathead minnows led to altered spawning behavior and reduced reproductive success (Brooks *et al.,* 2005). Similarly, investigations in zebrafish have shown fluoxetine-induced changes in sex steroid hormone levels, oxidative stress markers, and gene expression related to apoptosis and cell proliferation in gonadal tissue (Chen *et al.,* 2018).

The significant negative correlation between egg quantity and quality observed in this study further supports the hypothesis of a dose- or exposure-dependent trade-off (Ribeiro *et al.,* 2021). One plausible explanation is that fluoxetine accelerates oocyte release without sufficient maturation, leading to an increased number of eggs that are morphologically normal but developmentally compromised. The persistence of reduced egg viability in the post-treatment phase suggests either incomplete clearance of fluoxetine or long-lasting physiological alterations, such as epigenetic modifications or mitochondrial dysfunction, which may impair reproductive capacity beyond the exposure window.

Fluoxetine's endocrine-disrupting potential has been documented across species, and zebrafish models have been instrumental in uncovering its multi-level effects—from neuroendocrine control to ovarian follicle dynamics (Schreiber *et al.,* 2010). Studies have identified fluoxetine residues in aquatic ecosystems due to human pharmaceutical waste, raising concerns about its sublethal but significant effects on aquatic wildlife (Fick *et al.,* 2009). The current findings reinforce the ecological and toxicological relevance of fluoxetine as an environmental contaminant, capable of inducing reproductive disturbances even at low concentrations (3.2 µg/L in this study).

In terms of human relevance, while direct extrapolation from zebrafish to humans should be cautious, these results offer mechanistic insights into how SSRIs might impact female fertility. Clinical reports on SSRIs have yielded mixed results—some suggesting anovulation or menstrual irregularities, while others report no significant reproductive disruption (Bérard *et al.,* 2017). Animal models like zebrafish bridge this gap by enabling controlled, longitudinal observation of fertility outcomes under isolated pharmacological conditions (Kalueff *et al.,* 2014).

Notably, the zebrafish model used in this study offered several advantages, including high fecundity, transparent embryos, and established reproductive cycles, allowing clear visualization and quantification of developmental endpoints. However, limitations must be acknowledged. The small sample size (n=5 pairs), though standard for zebrafish fertility studies, may limit the generalizability of the results. Furthermore, the study did not assess hormonal levels, histopathological changes in gonads, or gene expression profiles, which could have provided deeper insights into underlying mechanisms.

Future studies should explore the dose-response relationship of fluoxetine on fertility outcomes and investigate the reversibility of its effects over longer recovery periods. Incorporating endpoints such as spawning latency, larval survival, oxidative stress biomarkers, and transcriptomic analysis of reproductive tissues could yield a more comprehensive understanding of fluoxetine's impact. Additionally, examining male fertility parameters in parallel could uncover potential sex-specific effects.

Conclusion

Fluoxetine exposure significantly increased egg production but reduced egg viability in female zebrafish, indicating a dual effect on reproductive physiology. These findings underscore the potential reproductive risks of fluoxetine in aquatic organisms and possibly in humans. Future studies should investigate underlying mechanisms, long-term effects, and transgenerational impacts to better inform environmental and pharmacological safety assessments.

Acknowledgments

The author would like to thank the M Mohammed Idris, Senior Principal Scientist, Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India, for guiding in the development of the Zebrafish Lab at our Institute.

References

Bérard, A, J.P. Zhao, O. Sheehy. 2017. Antidepressant use during pregnancy and the risk of major congenital malformations in a cohort of depressed pregnant women: an updated analysis of the Quebec Pregnancy Cohort. BMJ Open, 7(1): e013372.

Brooks, B.W., C.K. Chambliss, J.K. Stanley, A. Ramirez, K.E. Banks, R.D. Johnson, R.J. Lewis. 2005. Determination of select antidepressants in fish from an effluent-dominated stream. Environ Toxicol Chem, 24(2): 464–9.

Cassar, S., I. Adatto, J.L. Freeman, J.T. Gamse, I. Iturria, C. Lawrence, A. Muriana, R.T. Peterson, S. Van Cruchten, L.I. Zon. 2020. Use of Zebrafish in Drug Discovery Toxicology. Chem Res Toxicol, 33(1): 95-118.

Chen, W., X. Liu, S. Xu, Y. Zhao, X. Zhang, H. Liu. 2018. Fluoxetine disrupts sex hormone levels and expression of key genes involved in steroidogenesis and apoptosis in zebrafish (Danio rerio). Environ Pollut, 240: 342–50.

Dorelle, L.S., R.H. Da Cuña, G. Rey Vázquez, C. Höcht, A. Shimizu, G. Genovese, F.L. Lo Nostro. 2017. The SSRI fluoxetine exhibits mild effects on the reproductive axis in the cichlid fish Cichlasoma dimerus (Teleostei, Cichliformes). Chemosphere. 171: 370-378.

Fick, J., H. Söderström, R.H. Lindberg, C. Phan, M. Tysklind, D.G.J. Larsson. 2009. Contamination of surface, ground, and drinking water from pharmaceutical production. Environ Toxicol Chem, 28(12): 2522–7.

Hoo, J.Y., Y. Kumari, M.F. Shaikh, S.M. Hue, B.H. Goh. 2016. Zebrafish: A Versatile Animal Model for Fertility Research. Biomed Res Int, 2016:9732780.

Jing, E., K. Straw-Wilson. 2016. Sexual dysfunction in selective serotonin reuptake inhibitors (SSRIs) and potential solutions: A narrative literature review. Ment Health Clin, 6(4):191-196.

Kalueff, A.V., A.M. Stewart, R. Gerlai. 2014. Zebrafish as an emerging model for studying complex brain disorders. Trends Pharmacol Sci, 35(2): 63–75.

Li, J., W. Ge. 2020. Zebrafish as a model for studying ovarian development: Recent advances from targeted gene knockout studies. Mol Cell Endocrinol, 507:110778.

Maswood, N., J. Sarkar, L. Uphouse. 2008. Modest effects of repeated fluoxetine on estrous cyclicity and sexual behavior in Sprague Dawley female rats. Brain Res, 1245: 52-60.

Perez-Caballero, L., S. Torres-Sanchez, L. Bravo, J.A. Mico, E. Berrocoso. 2014. Fluoxetine: A case history of its discovery and preclinical development. Expert Opin Drug Discov, 9:567–578.

Reeves, K.W., O.I. Okereke, J. Qian, S.S. Tworoger, M.S. Rice, S.E. Hankinson. 2016. Antidepressant use and circulating prolactin levels. Cancer Causes Control, 27(7): 853-61.

Ribeiro, C., J. Saraiva, A. Florindo, A. Cruz, S.M. Monteiro. 2021. Antidepressant fluoxetine impacts the trade-off between reproductive output and offspring quality in zebrafish. Aquat Toxicol, 231:105718.

Ruiz-Santiago, C., C.V. Rodríguez-Pinacho, G. Pérez-Sánchez, E. Acosta-Cruz. 2024. Effects of selective serotonin reuptake inhibitors on endocrine system (Review). Biomed Rep.;21(3):128.

Schreiber, A.M., J.L. Specker, S.E. Perry. 2010. Neuroendocrine disruption: historical roots, current progress, and future directions. Gen Comp Endocrinol, 165(3):429–35.

Serré, C.M., B. Slowikowski, Y. Moon, M. Baker, E.M. Rinchik, N.G. Forger. 2021. The role of serotonin in regulation of the hypothalamic-pituitary-gonadal axis in mammals. Endocrinology, 162(8):bqab109.

Thompson, W.A., M.M. Vijayan. 2022. Antidepressants as Endocrine Disrupting Compounds in Fish. Front Endocrinol (Lausanne), 16(13):895064.

Weinberger, J.2nd., R. Klaper. 2014. Environmental concentrations of the selective serotonin reuptake inhibitor fluoxetine impact specific behaviors involved in reproduction, feeding and predator avoidance in the fish Pimephales promelas (fathead minnow). Aquat Toxicol, 151:77-83.

Zha, W., W. Hu, C. Ge, J. Chen, Z. Cao. 2024. Zebrafish as a model system for studying reproductive diseases. Front. Cell Dev. Biol. . 2:1481634.