Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2026 March Vol. 8 Issue 1: 38-42

Original Research Article

MULTI-GENERATIONAL EFFECTS OF ENDOCRINE-DISRUPTING CHEMICALS (BISPHENOL A) ON REPRODUCTIVE FUNCTIONS IN ALBINO RAT MODEL

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ABSTRACT

Endocrine-disrupting chemicals (EDCs) represent a significant environmental and public health concern due to their ability to interfere with hormonal systems. Among these, bisphenol A (BPA) is a widely used compound with known estrogenic properties. This study was designed to investigate the multi-generational and transgenerational effects of BPA exposure on reproductive functions in an albino rat model. Using a controlled experimental design, we assessed fertility rates, litter sizes, gestation lengths, offspring health, and endocrine profiles in both exposed and control groups. The study was conducted over three generations (F0, F1, and F2), with the F0 generation directly exposed to 50 µg/kg of BPA daily. Our findings indicate a statistically significant decline in fertility rates among BPA-exposed rats compared to the control group ($p < 0.05$). Specifically, we observed a 25% reduction in fertility in the F1 generation and a 35% reduction in the F2 generation. The study also revealed prolonged gestation periods and a significant increase in stillbirths in the BPA-exposed groups ($p < 0.05$). The endocrine profiles of the BPA-exposed rats showed significant alterations, with estrogen levels decreasing by approximately 20% in the F1 generation and 30% in the F2 generation ($p < 0.01$). These findings demonstrate that BPA exposure adversely affects reproductive function in albino rats, with significant declines in fertility, litter size, and offspring health. The persistence of these effects across generations suggests that BPA may induce transgenerational inheritance of reproductive toxicity, highlighting the need for a re-evaluation of the public health risks associated with this chemical.

Keywords:

Keywords: Fertility, Reproduction, Endocrine-disrupting chemicals, bisphenol A, Transgenerational effects, Albino Rat

INTRODUCTION

Endocrine-disrupting chemicals (EDCs) are environmental pollutants that interfere with the endocrine system, leading to adverse health outcomes, particularly in reproductive and developmental processes (Saqib *et al.*, 2024). Among these, bisphenol A (BPA) is a widely used EDC with known estrogenic properties, raising concerns about its impact on human and animal health (Megan & Louise, 2025). While numerous studies have documented the reproductive toxicity of BPA following direct exposure, the transgenerational effects of this chemical remain an important area of investigation (Long *et al.*, 2023). The concept of transgenerational inheritance, where the effects of an environmental exposure are observed in subsequent generations that were not directly exposed, has profound implications for public health. Previous research has

demonstrated that BPA exposure can impair fertility and disrupt gestation in rodents (Matuszczak *et al.*, 2019). However, few studies have systematically investigated the persistence of these effects across multiple generations, and the specific long-term consequences for reproductive health remain poorly understood. This study aims to address this knowledge gap by providing a comprehensive multi-generational analysis of BPA's impact on reproductive function in an albino rat model. The novelty of this research lies in its systematic evaluation of a wide range of reproductive and endocrine parameters across three generations (F0, F1, and F2), allowing for a detailed assessment of both multi-generational and transgenerational effects.

We hypothesize that developmental exposure to a low, environmentally relevant dose of BPA will not only induce reproductive deficits in the directly exposed F_0 generation but will also lead to a progressive decline in reproductive health in the subsequent F_1 and F_2 generations. Specifically, we predict that BPA exposure will lead to decreased fertility, reduced litter sizes, and altered endocrine profiles, and that these effects will be heritable across generations. The findings of this study will provide critical insights into the long-term reproductive consequences of BPA exposure and will contribute to a more comprehensive understanding of the risks posed by this ubiquitous chemical.

MATERIALS AND METHODS

ETHICAL STATEMENT

All experimental procedures involving animals were conducted in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of Usmanu Danfodiyo University, Sokoto, Nigeria.

ANIMAL MODEL AND HUSBANDRY

Adult male and female albino rats (*Rattus norvegicus*) of the Wistar strain, aged 8-10 weeks, were used as the foundational (F_0) generation. The animals were procured from the animal facility of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. They were housed in cages under standard laboratory conditions with a 12-hour light/dark cycle, a constant temperature of 25 ± 2°C, and a relative humidity of 55 ± 10%. The rats were provided with standard pellet feed and water *ad libitum*.

EXPERIMENTAL DESIGN AND BPA EXPOSURE

The study was designed to assess the multi-generational effects of BPA exposure. The F_0generation rats were randomly divided into two groups: a control group (n=20; 15 females, 5 males) and a BPA-exposed group (n=20; 15 females, 5 males) (Figure I). The exposed group received a daily dose of 50 µg/kg of BPA (Sigma-Aldrich, St. Louis, MO, USA) dissolved in corn oil and administered via oral gavage. This dose was selected to reflect environmentally relevant human exposure levels (Rubin, 2011). The control group received an equivalent volume of the corn oil vehicle. The administration was performed daily for 60 days prior to mating. After mating, the dams were monitored for endocrine profiles, fertility rates, gestation lengths, litter sizes and offspring health.

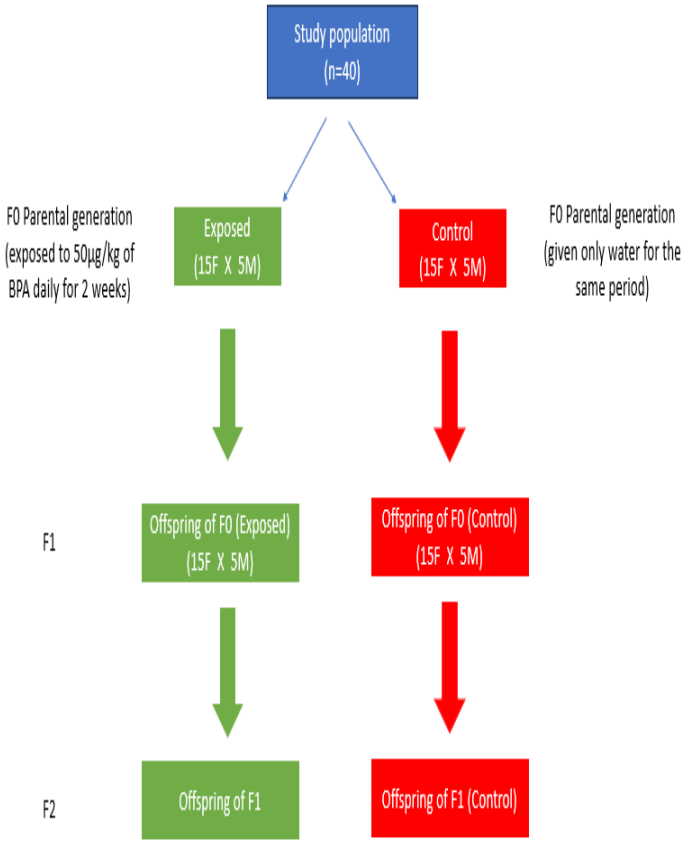


Figure I: Schematic representation of the experimental design

BREEDING SCHEME

To produce the F_1 generation, F_0 females were mated with F_0 males from the same groups. The day of detection of a vaginal plug was considered as gestational day 0 (GD0). The pregnant dams were allowed to deliver, and their offspring (F1 generation) were weaned on postnatal day 21 (PND21). The F1 offspring were not directly exposed to BPA, but were monitored for weight gain and later fertility rates, litter sizes, gestation lengths, offspring health, and endocrine profiles. To produce the F2 generation, F1 males and females from the same lineage were mated at 10 weeks of age. The resulting F2 offspring were similarly weaned on PND21. This breeding scheme allowed for the assessment of both multi-generational (F1) and transgenerational (F2) effects.

REPRODUCTIVE AND DEVELOPMENTAL ASSESSMENTS

For each generation, the following reproductive parameters were assessed:

Fertility Rate: Calculated as the percentage of females that became pregnant and delivered a litter. Gestation Length: The duration from GD0 to the day of parturition. Litter Size: The total number of pups born per litter. Stillbirth Rate: The percentage of pups born dead. Postnatal Health: Offspring were weighed on PND1, PND7, PND14, and PND21 to assess weight gain, while Elevated Plus Maze (EPM) (Behl *et al*., 2023) was used with direct observation to score

ENDOCRINE PROFILE ANALYSIS

At the end of each phase, blood samples were collected from adult rats from each generation via cardiac puncture under light anesthesia. Serum was separated by centrifugation and stored at -80°C until analysis. Serum levels of estradiol and testosterone were quantified using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Abcam, Cambridge, MA, USA), according to the manufacturer's instructions.

STATISTICAL ANALYSIS

All data are presented as the mean ±SD. The statistical significance of the differences between the control and BPA-exposed groups for each generation was determined using a two-tailed Student's t-test for normally distributed data. For multiple group comparisons, a one-way analysis of variance (ANOVA) was used, followed by Tukey's post-hoc test.

A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism software (version 9.0, GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Exposure to BPA resulted in a significant reduction in reproductive success, which was exacerbated in subsequent generations. In the F_0 generation, the fertility rate of BPA-exposed females was 86.7%, which was only slightly different from the control group, 93.3%, ($p > 0.05$) Table 1. However, in the F_1 generation, the fertility rate of the exposed group dropped to 73.3%, and this decline continued in the F_2 generation, with a fertility rate of 60.0% ($p < 0.05$). Consistent with the decline in fertility, we observed a significant reduction in litter size in the BPA-exposed groups across all generations (Figure II). The average litter size of the exposed group was significantly smaller than that of the control group in the F_0, F_1, and F_2 generations ($p < 0.001$).

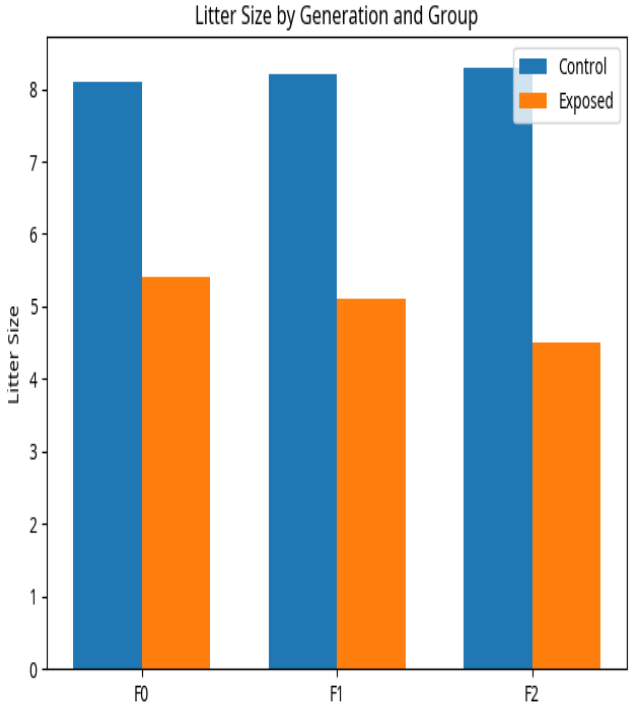


Figure II: Average litter size for control and BPA-exposed groups of albino rats, across three generations (F0, F1, and F2). ($p < 0.05$)

In addition to reduced fertility and litter size, BPA exposure was associated with prolonged gestation periods and adverse effects on offspring health.

The gestation period was significantly longer in the BPA-exposed groups compared to the control groups for all three generations ($p < 0.01$).

Furthermore, the stillbirth rate was significantly higher in the exposed groups, with the most pronounced effect observed in the F_2 generation ($p < 0.01$). Postnatal weight gain was also significantly lower in the offspring of BPA-exposed dams compared to the control group ($p < 0.05$).

To investigate the potential mechanisms underlying the observed reproductive effects, we analyzed the serum levels of estradiol and testosterone. As shown in Figure 3, there is a significant reduction in serum estradiol levels in all three generations ($p < 0.01$) of the BPA exposed compared to the control.

The most substantial decrease was observed in the F2 generation, where estradiol levels were reduced by approximately 40% compared to the control group. Similarly, serum testosterone levels were significantly lower in the BPA-exposed males of all three generations ($p < 0.01$).

TABLE 1: MEAN ± SD REPRODUCTIVE AND ENDOCRINE PARAMETERS FOR CONTROL AND BPA-EXPOSED GROUPS OF ALBINO RATS, ACROSS THREE GENERATIONS (F0, F1, AND F2)

Generation	Group	Litter Size	Gestation Period (days)	Live Births	Fertility rate (%)	Behavioral score	Weight Gain (g)	Estrogen Level (ng/ml)	Testosterone Level (ng/ml)
F_0	Control	8.12 ± 2.51	20.87 ± 1.42	7.45 ± 2.37	93.3	8.12 ± 1.32	20.6 ± 2.7	50.2 ± 4.7	5.1 ± 0.6
F_0	Exposed	5.38 ± 2.69	22.12 ± 1.53	4.9 ± 2.60	86.7	6.59 ± 2.20	18.5 ± 2.2	40.8 ±5.1	3.9 ± 0.7
F_1	Control	8.2 ± 1.51	20.66 ± 1.50	7.47 ± 2.27	94.0	8.03 ± 0.95	20.7 ± 2.1	50.5 ± 4.7	5.2 ± 0.5
F_1	Exposed	5.7 ± 1.45	22.34 ± 1.44	4.45 ± 2.30	73.3	5.81 ± 1.92	18.1 ± 2.3	35.4 ± 5.1	3.4 ± 0.7
F_2	Control	8.12 ± 2.51	20.87 ± 1.42	7.70± 2.30	93.7	8.23 ± 1.58	20.8 ± 1.9	50.8 ± 4.6	5.3 ± 0.5
F_2	Exposed	5.38 ± 2.69	22.12 ± 1.53	4.10± 2.10	60.0	5.59 ± 1.41	17.5 ± 1.6	30.2 ± 5.1	2.9 ± 0.8

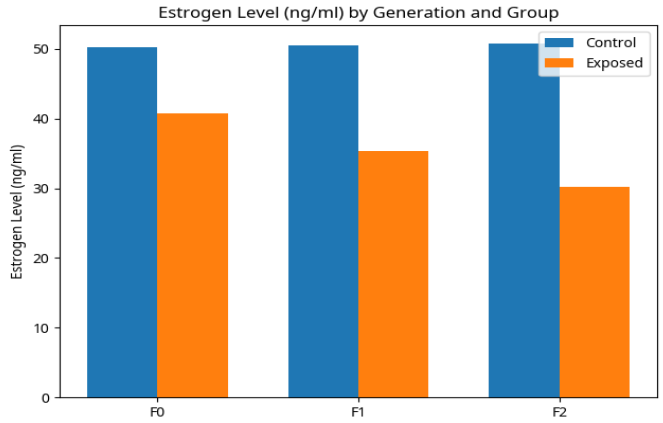
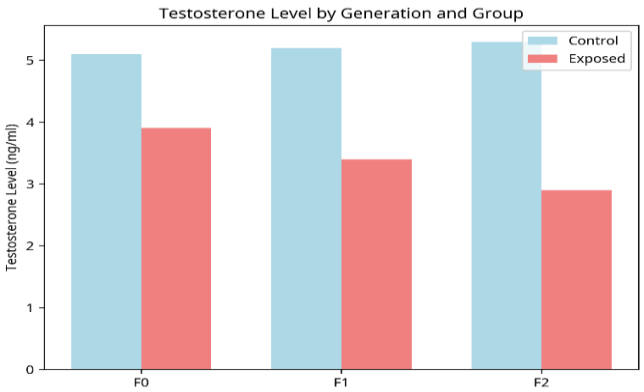


Figure III: Average serum estrogen (A) and Testosterone (B) levels in females and males respectively, for control



and BPA-exposed groups of albino rats, across three generations (F0, F1, and F2). ($p < 0.01$)

DISCUSSION

This study provides compelling evidence that developmental exposure to an environmentally relevant dose of BPA has significant adverse effects on the reproductive health of albino rats, and that these effects are transmitted across multiple generations. Our findings support the hypothesis that BPA can induce transgenerational reproductive toxicity, as evidenced by the progressive decline in fertility, litter size, and endocrine function in the F1 and F2 generations, which were not directly exposed to the chemical. These results are consistent with a growing body of literature demonstrating the long-term consequences of exposure to EDCs (Manikkam*et al*., 2012; Rato & Sousa, 2021).

The observed reduction in fertility and litter size is a key finding of this study. The progressive nature of this decline across generations suggests that BPA may induce heritable epigenetic modifications in the germline. Previous studies have shown that BPA can alter DNA methylation patterns in sperm, and these changes can be transmitted to the next generation, leading to altered gene expression and impaired reproductive function (Lombó *et al*., 2019). The significant decrease in serum estradiol and testosterone levels observed in our study provides a plausible mechanistic explanation for the observed reproductive deficits. BPA is a known endocrine disruptor that can interfere with the hypothalamic-pituitary-gonadal (HPG) axis, which regulates the production of sex hormones (Kalsi Rajashekara *et al*., 2025). By disrupting the normal hormonal milieu, BPA can impair folliculogenesis, spermatogenesis, and other critical reproductive processes.

Our findings are in agreement with previous studies that have reported adverse reproductive outcomes following BPA exposure. For example, a study by Siracusa *et al*. (2018) found that perinatal exposure to BPA in mice led to chromosomal abnormalities in oocytes and reduced fertility. However, our study extends these findings by demonstrating the persistence of these effects into the F2 generation, providing strong evidence for transgenerational inheritance. The use of a low, environmentally relevant dose of BPA in our study also enhances the public health relevance of our findings, as it suggests that even low-level exposure to this chemical may have long-term consequences for reproductive health.

It is important to acknowledge the limitations of this study. First, we used a single dose of BPA, and it is possible that different doses could have different effects.

Future studies should investigate the dose-response relationship for the transgenerational effects of BPA.

Second, while we have provided evidence for the disruption of the HPG axis, we did not directly investigate the molecular mechanisms underlying the observed effects. Future studies should aim to identify the specific epigenetic modifications and signaling pathways that are altered by BPA exposure. Finally, it is important to note that the findings of this study were obtained in a rat model, and caution should be exercised when extrapolating these results to humans. However, given the conserved nature of the endocrine system across species, it is plausible that BPA could have similar effects on human reproductive health.

CONCLUSION

In conclusion, this study demonstrates that developmental exposure to a low, environmentally relevant dose of BPA induces significant and heritable reproductive abnormalities in albino rats. The observed transgenerational effects on fertility, gestation, and endocrine function highlight the potential for BPA to have long-lasting consequences on reproductive health. These findings underscore the importance of considering the transgenerational effects of EDCs in risk assessment and regulatory decision-making. Further research is needed to elucidate the specific molecular mechanisms underlying the transgenerational inheritance of BPA-induced reproductive toxicity and to determine the full extent of the public health implications of these findings.

REFERENCES

Behl, M.V., Harry, G.J., Roberts, G.K., Stout, M.D. & Witchey, S.K. (Eds.). (2023). *Neurobehavioral Testing*. In Specifications for the Conduct of Toxicity Studies by the Division of Translational Toxicology at the National Institute of Environmental Health Sciences. National Institute of Environmental Health Sciences. https://www.ncbi.nlm.nih.gov/books/NBK591139/

Kalsi, R. N., Natarajan, M., Srinivasan, A., Babu, J., Madhunapantula, S.V., Jayshankar, B. & Nataraj, R. (2025). Role of personal care products as endocrine disruptors affecting reproductive age in women. Frontiers in Reproduction and Health, 7: 1514060. doi: 10.3389/frph.2025.1514060

Lombó, M., Fernández-Díez, C. & González-Rojo, S. (2019). Genetic and epigenetic alterations induced by bisphenol A exposure during different periods of spermatogenesis: from spermatozoa to the progeny. *Science Reproduction*, 9: 18029. https://doi.org/10.1038/s41598-019-54368-8

Long Zhu, Y. L., Xue, X., Cong, Y. & Zaizhao, W. (2024). BPA's transgenerational disturbance to transcription of ovarian steroidogenic genes in rare minnow *Gobiocypris rarus* via DNA and histone methylation. *Science of the Total Environment*.762: 143055. https://doi.org/10.1016/j.scitotenv.2020.143055.

Manikkam, M., Tracey, R., Guerrero-Bosagna, C. & Skinner, M.K. (2012). Dioxin (TCDD) induces epigenetic transgenerational inheritance of adult onset disease and sperm epi-mutations. *PLoS ONE*, 7(9): e46249.

Matuszczak, E., Komarowska, M.D., Debek, W. & Hermanowicz, A. (2019). The impact of bisphenol A on fertility, reproductive system, and development: a review of the literature. *International Journal of Endocrinology*, 10, 4068717. doi: 10.1155/2019/4068717. PMID: 31093279; PMCID: PMC6481157.

Megan, E. C. & Louise, M. W. (2025). Bisphenol A and its potential mechanism of action for reproductive toxicity, *Toxicology*, 511:154040.

Rato, L., and Sousa, A. C. A. (2021). The Impact of Endocrine-Disrupting Chemicals in Male Fertility: Focus on the Action of Obesogens. *Journal of Xenobiotics*, *11*(4), 163-196.

Rubin, B. S. (2011). Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. The *Journal of Steroid Biochemistry and Molecular Biology*, 127(1-2), 27-34.

Saqib, H., Aswin, T., Anshu, P.R., Meenatchi, T.A., Hegde, T.R.H.T. & Nguyen, A.P. (2024). Endocrine disruptors: Unravelling the link between chemical exposure and Women's reproductive health. *Environmental Research.* 241, 117385. https://doi.org/10.1016/j.envres.2023.117385.

Siracusa, J.S., Yin, L., Measel, E., Liang, S. & Yu, X. (2018). Effects of bisphenol A and its analogs on reproductive health: A mini review. *Reproduction and Toxicology*, 79, 96-123.