MSI Journal of
Medicine and Medical Research (MSIJMMR)

Impact of Short-Term Formalin Exposure on Follicular Phase Reproductive Hormones Among Students at the Nnewi Campus Running head; Short-Term Formalin Exposure and Reproductive Hormones

OZURUOKE, Donatus F. N.[1], IHIM, Augustine Chinedu[2*], IFEH, Chinwoke Ifeh[2], EDEH, Ini[3], IKWELLE, Tochukwu Anthony[2], OBI, Collins Uchechukwu[1]

[1]Department of Medical Laboratory Science, Faculty of Medical and Health Sciences, Newgate University, Minna.

[2*]Department of Clinical Chemistry, Medical Laboratory Science Faculty, Nnamdi Azikiwe University, Awka, Nigeria.

[3]Medical Laboratory Science Council of Nigeria.

*** Correspondence:** IHIM, Augustine Chinedu

The authors declare that no funding was received for this work.

OPEN ACCESS

Received: 10-January-2026
Accepted: 25-February-2026
Published: 01-March-2026

This article is published in the **MSI Journal of Medicine and Medical Research (MSIJMMR)**
ISSN 3049-1401 (Online)

The journal is managed and published by MSI Publishers.

Volume 3, Issue 1 (Jan-Apr), 2026

ABSTRACT: Background: Formaldehyde is widely used as a preservative in cadaveric dissection laboratories, resulting in routine exposure of medical students during anatomical training. Despite its extensive use, concerns persist regarding its potential endocrine and cardiovascular effects, particularly following short-term exposure.

Objectives: This study assessed the effect of short-term (3-hour) formalin exposure on serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and blood pressure among female medical students at Nnamdi Azikiwe University, Nnewi Campus.

Methods: A cross-sectional, pre–post exposure study was conducted among 45 female medical students aged 18–35 years. Baseline blood samples collected prior to cadaveric dissection served as pre-exposure controls, while post-exposure samples were obtained after a 3-hour formalin exposure session. Serum FSH and LH levels were quantified

using enzyme-linked immunosorbent assay (ELISA). Blood pressure was measured using an automated digital sphygmomanometer (OMRON 907). Data were analyzed using paired t-tests and Pearson correlation analysis, with statistical significance set at $p < 0.05$.

Results: A significant reduction was observed in mean serum luteinizing hormone levels following formalin exposure (14.39 ± 11.23 IU/L) compared with baseline values (17.51 ± 16.10 IU/L) ($p < 0.05$). Systolic blood pressure increased significantly post-exposure relative to baseline ($p < 0.05$). A weak but significant negative correlation was found between post-exposure serum FSH levels and diastolic blood pressure ($r = -0.374$, $p = 0.011$).

Conclusion: Short-term formalin exposure was associated with reduced serum LH levels and increased systolic blood pressure in female medical students. These findings suggest that even brief exposure may disrupt hypothalamic–pituitary–gonadal axis regulation, potentially predisposing exposed individuals to hypoleutinism and hypogonadotropic hypogonadism. Enhanced exposure control measures and routine physiological monitoring are recommended in anatomy laboratories.

Keywords: *Formalin, Luteinizing hormone, Follicle-stimulating hormone, Hypogonadism, Hypoluteinism*

INTRODUCTION

The female reproductive system experiences hormonal and physiological fluctuations cyclically to prepare for conception and pregnancy. This cycle is known as menstrual cycle and it is necessary for preparation of the uterus for implantation of eggs and hormonal regulation. (Thiyagarajan et al., 2019).

Menstrual cycle is a complex and recurring process by which the female reproductive system prepares for potential pregnancy and it comprises phases like follicular phase, ovulatory phase and luteal phase (Patricio et al., 2018). The follicular phase begins on the first day of the menstruation and end with release of egg (ovulation). The duration is 14days of a 28-days cycle with most cycle lengths

between 25 to 30 days (Reed et al., 2018). The menstrual cycle, especially the follicular phase, is regulated by hormones that oversee the development of ovarian follicles. This process is influenced by key hormones including Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and oestradiol (Nanjappa et al., 2017).

Exposure to endocrine-disrupting chemicals, like formaldehyde, in both environmental and occupational settings has been shown to disrupt hormonal balance and reproductive function (Zolotnik, 2016). Formaldehyde was initially discovered by August Wilhelm von Hofmann, a prominent British chemist, in the year 1856 (Dixit, 2008). It is a widely used chemical in educational settings, particularly in departments of Anatomy to preserve cadavers. Formaldehyde is classified as a human carcinogen and is linked to various acute and chronic health effects, including irritation of the eyes, skin, and respiratory system (IARC, 2006). The exposure of this compound has been shown to generate oxidative stress, leading to cellular and tissue damage, including potential impacts on the endocrine system, particularly in disrupting the hormonal balance essential for reproduction (Nampoothiri *et al*., 2015), and has also been linked to hormonal imbalances, particularly affecting the follicular phase hormones, Follicle-Stimulating Hormone (FSH) and luteinizing Hormone (LH), crucial for reproductive health. This study aims to investigate the implication of short-term formaldehyde exposure on follicular phase hormones (FSH and LH) in female students.

MATERIALS AND METHOD

Study Site

The study was conducted in the Anatomy Laboratory of the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Okofia, Nnewi Campus, Anambra State, Nigeria.

Study Design and Population

This was a longitudinal study engaged with Pretest–posttest observational study involving female medical students aged 18–30 years who were routinely exposed to formalin during dissection classes in the Anatomy Laboratory, College of Health

Sciences, Nnewi Campus. A total of 45 participants were recruited. Baseline blood samples were collected prior to short-term formalin exposure, followed by post-exposure sample collection after a 3-hour dissection session.

Blood sampling was conducted during the follicular phase of the menstrual cycle, defined as days 1–14 of a typical 28-day cycle. For optimal assessment of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), samples were collected within this window both before and after formalin exposure to evaluate exposure-related hormonal changes. Prior to sample collection, participants completed a structured questionnaire to obtain relevant demographic, menstrual, and health-related information.

Inclusion Criteria

Eligible participants were female students enrolled in the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, within the reproductive age range of 18–35 years. Participants were required to be in the follicular phase of their menstrual cycle, have regular menstrual cycles (21–35 days), and be exposed to formalin for a duration of 3 hours during dissection classes. Only participants not using hormonal contraceptives or hormone-altering medications and without known endocrine disorders such as polycystic ovary syndrome (PCOS) or thyroid disease were included. Written informed consent was mandatory for participation.

Sample Size Determination

Sample size was calculated using G*Power software version 3.1.9.4 (Universität Düsseldorf, Germany). Analysis for differences between two dependent means (matched pairs) was performed using an alpha level of 0.05, statistical power of 0.95, and an effect size of 0.05. The calculated minimum sample size was 45 participants, which provided 95% power to detect a significant difference at a significance level of 0.05.

Ethical Approval

Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medical Laboratory Science, Nnamdi Azikiwe University (Reference No.:

FMLS/REC/025/075). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki of the World Medical Association. All participants' information was handled with strict confidentiality throughout the study.

Informed Consent

Written informed consent was obtained from all participants prior to enrolment.

Anthropometric Measurements and Body Mass Index

Height and weight were measured using a standard stadiometer and calibrated weighing scale prior to formalin exposure. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Overweight was defined as BMI $\geq$ 25 kg/m^2, as previously described (Ihim et al., 2017).

Blood Pressure Measurement

Blood pressure was measured using an automated sphygmomanometer (OMRON 907, Hoofddorp, Netherlands). Two readings were taken from the right arm at heart level, with a 60-second interval between measurements. The average systolic and diastolic values were recorded. Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg (Ihim et al., 2017).

Blood Sample Collection and Processing

Two milliliters of venous blood were collected aseptically from each participant before and after formalin exposure via the antecubital vein using minimal stasis and a sterile plastic syringe into plain sample bottles. Samples were allowed to clot and then centrifuged at 3000 rpm for 10 minutes. Serum was separated and used for the determination of LH and FSH. Samples not analyzed immediately were stored at −20°C until analysis.

Luteinizing Hormone Assay

Serum LH levels were determined using an enzyme immunoassay method as previously described (Ihim et al., 2017).

Principle of the Assay

The assay employed a two-step sandwich ELISA technique using two highly specific monoclonal antibodies. One antibody specific to LH was immobilized on the microplate, while the second antibody, conjugated to horseradish peroxidase (HRP), bound to a different epitope of LH. Following incubation and washing steps, substrate addition resulted in a colorimetric reaction proportional to the LH concentration, which was measured spectrophotometrically at 450 nm.

Assay Procedure

All reagents were equilibrated to room temperature prior to use. Calibrators, controls, and samples (25 µL each) were pipetted into designated wells in duplicate, followed by the addition of assay buffer. After incubation and washing, HRP conjugate was added and incubated. Following a second wash, TMB substrate was added, and the reaction was stopped using stop solution. Absorbance was read at 450 nm within 20 minutes.

Follicle-Stimulating Hormone Assay

FSH levels were measured using a solid-phase sandwich ELISA according to the manufacturer's instructions (Chihara et al., 2020 ; Ihim et al., 2024)

Principle of the Assay

Microtiter wells coated with monoclonal anti-FSH antibodies captured endogenous FSH from serum samples. An HRP-conjugated secondary antibody bound to the antigen–antibody complex. Color development following substrate addition was directly proportional to the FSH concentration and measured at 450 nm.

Assay Procedure

Standards, controls, and samples (25 µL) were dispensed into appropriate wells, followed by enzyme conjugate addition and incubation. Wells were washed thoroughly, substrate was added, and the reaction was terminated with stop solution. Absorbance was measured at 450 ±10 nm within 10 minutes.

Statistical Analysis

Data were analysed using paired Student's *t*-tests to compare pre- and post-exposure hormone and blood pressure levels. Pearson's correlation coefficient was used to assess associations between study parameters. Statistical significance was set at $p < 0.05$.

RESULTS

Table 4.1: Mean serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels of participants before and after short-term (3-hour) formalin exposure (mean ± SD)

There was no statistically significant difference in mean serum FSH concentrations following short-term formalin exposure (14.994 ± 17.426 IU/L) compared with baseline values (14.043 ± 9.205 IU/L) ($p > 0.05$).

In contrast, mean serum LH concentrations showed a significant reduction post-exposure (14.385 ± 11.230 IU/L) compared with baseline levels (17.507 ± 16.100 IU/L) ($p < 0.05$).

Table 4.1: Mean serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels of participants before and after short-term (3-hour) formalin exposure (mean ± SD)

Variables	**Pre-formalin exposure (n=45)**	**Post-formalin exposure (n=45)**	**t-value**	**p-value**
Luteinizing Hormone (IU/L)	17.507 ± 16.100	14.385 ± 11.2301	2.168	0.036*
Follicle Stimulating Hormone (IU/L)	14.043 ± 9.205	14.994 ± 17.426	-0.348	0.729

*Statistically significant at p<0.05

Table 4.2: Mean systolic and diastolic blood pressure of participants before and after short-term (3-hour) formalin exposure (mean ± SD).

There was no statistically significant difference in mean diastolic blood pressure before (76.822 ± 7.744 mmHg) and after (77.911 ± 7.856 mmHg) short-term formalin exposure ($p > 0.05$).

In contrast, mean systolic blood pressure showed a statistically significant increase following exposure, rising from 111.511 ± 9.367 mmHg at baseline to 112.844 ± 9.672 mmHg post-exposure ($p < 0.05$).

Table 4.2: Mean systolic and diastolic blood pressure of participants before and after short-term (3-hour) formalin exposure (mean ± SD).

Variables	**Pre-formalin exposure (n=45)**	**Post-formalin exposure (n=45)**	**t-value**	**p-value**
Diastolic Blood Pressure (mmHg)	76.822± 7.744	77.911 ± 7.856	-3.117	0.060
Systolic Blood Pressure (mmHg)	111.511 ± 9.367	112.844 ± 9.672	-2.895	0.030*

*Statistically significant at p<0.05

Table 4.3: Association between post-exposure serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels and selected cardiometabolic parameters following short-term (3-hour) formalin exposure.

Post-exposure analysis showed no significant association between LH levels and body mass index (BMI) ($r = 0.097$, $p = 0.528$), systolic blood pressure (SBP) ($r = −0.115$, $p = 0.453$), or diastolic blood pressure (DBP) ($r = −0.290$, $p = 0.540$).

Similarly, FSH levels were not significantly associated with BMI ($r = −0.063$, $p = 0.528$) or SBP ($r = −0.100$, $p = 0.948$). However, a moderate negative correlation was observed between FSH levels and DBP ($r = −0.374$, $p = 0.011$).

Table 4.3: Association between post-exposure serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels and selected cardiometabolic parameters following short-term (3-hour) formalin exposure.

Parameters	**BMI (kg/m^2)**	**DBP (mmHg)**	**SBP (mmHg)**
Luteinizing Hormone, LH (IU/L)	r = 0.970 p = 0.528	r = -0.290 p = 0.540	r = -0.115 p = 0.453
Follicle Stimulating Hormone, FSH (IU/L)	r = -0.63 p = 0.681	r = -0.374 p = 0.011	r = -0.100 p = 0.948

*Statistically significant at p<0.0

DISCUSSION

Medical students and staff working in anatomy laboratories constitute a high-risk population for potential adverse health effects associated with formaldehyde exposure (Noha and Madiha, 2017). This study evaluates the serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in female students who, although previously exposed to formalin during routine academic activities, experienced an additional controlled short-term exposure of 3 hours at the Nnewi Campus. This design allowed assessment of acute hormonal responses to formalin exposure during the follicular phase of the menstrual cycle.

Findings from this study demonstrated no statistically significant difference in mean serum FSH levels following short-term formalin exposure compared with baseline values. This result contrasts with the findings of Ihim et al. (2017), who reported a significant increase in FSH levels following short-term formalin exposure in male subjects. The discrepancy may be explained by differences in sex, hormonal regulation, and reproductive physiology. In males, alterations in Sertoli cell function following toxic exposure can reduce inhibin secretion, thereby diminishing negative feedback on the pituitary gland and leading to elevated FSH levels (Sahah et al., 2021). The absence of a similar response in the present female cohort suggests possible sex-specific endocrine responses to formaldehyde exposure or differential sensitivity of regulatory pathways.In contrast, a statistically significant reduction in mean serum LH levels was observed following short-term formalin exposure. This finding is consistent with the study by Lutvia et al. (2023), which demonstrated reduced levels of luteinizing hormone-releasing hormone (LHRH) in female rats exposed to formalin. LHRH plays a critical role in stimulating LH secretion from the anterior pituitary, and suppression of its expression or receptor activity may result in decreased circulating LH levels. The observed decline in LH in this study suggests that formalin exposure may interfere with hypothalamic–pituitary signalling, potentially impairing follicular maturation and ovulatory processes. Such disruption could have implications for reproductive function if exposure is repeated or prolonged.

No significant difference was observed in mean diastolic blood pressure following the 3-hour formalin exposure. However, a statistically significant increase in systolic blood pressure was recorded post-exposure. These findings are partially consistent with Okonkwo et al. (2018), who reported a significant increase in systolic blood pressure and a non-significant change in diastolic blood pressure following prolonged formaldehyde exposure. Augenreich et al. (2020) observed no significant changes in either systolic or diastolic blood pressure following a shorter exposure duration of 90 minutes. Collectively, these findings suggest that the cardiovascular response to formaldehyde may be influenced by exposure duration and intensity, with systolic blood pressure appearing more sensitive to acute exposure.

Correlation analysis revealed a moderate negative association between FSH levels and diastolic blood pressure. This observation aligns with findings by Wang et al. (2017), who reported associations between FSH and cardiometabolic risk markers in postmenopausal women. Although the mechanistic link between FSH and blood pressure regulation is not fully understood, emerging evidence suggests that FSH receptors are expressed in vascular endothelial cells, where FSH may influence vascular tone and endothelial function. Additionally, interactions between FSH, estrogen, and metabolic pathways may contribute to blood pressure regulation. While the present study population differs from postmenopausal cohorts, the observed association supports a potential role of FSH in cardiovascular physiology that warrants further investigation.

Overall, these findings suggest that short-term formalin exposure may selectively affect reproductive and cardiovascular parameters, particularly LH secretion and systolic blood pressure, even in the absence of significant changes in FSH levels. Further studies with larger sample sizes and longer follow-up periods are needed to elucidate the underlying mechanisms and long-term health implications of formalin exposure in occupational and academic settings.

CONCLUSION

This study demonstrates that short-term (3-hour) exposure to formalin among female medical students is associated with selective alterations in reproductive and

cardiovascular parameters. While no significant change was observed in serum follicle-stimulating hormone (FSH) levels following exposure, a significant reduction in serum luteinizing hormone (LH) concentrations was recorded, suggesting that formalin may acutely disrupt hypothalamic–pituitary–gonadal axis regulation during the follicular phase of the menstrual cycle. This finding highlights LH as a potentially more sensitive endocrine marker of short-term formalin exposure in females.

Further research

Given the **limited sample size (n = 90)** and the **short duration of exposure**, further research is warranted. Future studies should involve **larger, multi-centre cohorts**, assess the **effects of varying exposure durations and concentrations**, and include **longitudinal follow-up** to determine potential cumulative or long-term reproductive and cardiovascular consequences. Additionally, incorporating **other reproductive hormones, oxidative stress markers, and environmental exposure assessments** would provide a more comprehensive understanding of the biological impact of formalin exposure among medical students.

REFERENCES

1. Augenreich, M., Stickford, J., Stute, N., Koontz, L.., Cope, J., Bennett, C. and Ratchford, S.M. (2020). Vascular dysfunction and oxidative stress caused by acute formaldehyde exposure in female adults. *American Journal of Physiology*; 319(6).

2. Chihara, K., Hattori, N., Matsuda, T., Murasawa, S., Daimon, M. and Shimatsu, A. (2020). Procedures for the diagnosis of macro-follicle stimulating hormone (FSH) in a patient with high serum FSH concentrations. *Clinical Chemistry and Laboratory Medicine (CCLM)*; 58(2):40-43.

3. Dixit, D., 2008. Role of standardized embalming fluid in reducing the toxic effects of formaldehyde. Indian Journal of Forensic Medicine &Toxicology; *2*(1):33-39.doi:https://doi.org/10.26655/JMCHEMSCI.2023.10.24.

4. Ihim, C.A., Chukwuemeka, O.E., Ndumnworo, O.D., Kalu, A.U. and Victor, O. (2017). Effect of short-term exposure to formalin on male reproductive hormones of students in Nnewi. IOSR *Journal of Dental and Medical Sciences*; 16:33-36.

5. Ihim, A.C., Onyenekwe, C.C, Eze, N.N, Obi, P.C., Osakue, N., Awalu, J.C., Ikwelle, T.A. (2024) Evaluation of Some Hormones Total Antioxidant Capacity and Malondialdehyde Levels in Polycystic Ovarian Syndrome Women attending the gynecology Clinic at Nnewi, Journal of Drug Delivery and Therapeutics. 14(5):108-112 *DOI: http://dx.doi.org/10.22270/jddt.v14i5.6539*

6. International Agency for Research on Cancer (IARC). (2006). Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 88, 39–325

7. Lutviai, K. and Purwanto, B. (2023). Effect of Formalin Exposure on Antral Follicle Development Disorders through Decreased Gonadotropin Hormone Regulation. *Journal of Medicinal and Chemical Sciences*, [online] *6*:2502–2509.

8. Nampoothiri, L.P., Sreekumar, R., & Retnakumari, A. (2015).Formaldehyde crosses the human placenta and affects human trophoblast differentiation and hormonal functions. *Public Library of Science ONE*, 10(7), e0133330.

9. Nanjappa, M.K., Cooke, P.S. and Hess, R.A., 2017**.** Estrogens in male physiology. *Physiological Reviews*, 97(3):995–1043.

10. Noha, S.M., and Madiha, A.E., (2017). Toxic effects of formalin-treated cadaver on medical students, staff members, and workers in the Alexandria Faculty of Medicine. Alexandria Journal of Medicine; 53 (2017) 337–34.

11. Okonkwo, C.O., Metu, S.C., Maduka, S.O. and Oguaka, V.N., 2018. Effects of formaldehyde inhalation on cardiopulmonary functions on medical students of College of Health Sciences, Nnamdi Azikiwe University during dissection classes. *Asian Journal of Pharmaceutical and Biological Research*, 8(1):68–72.

12. Patricio, B.P. and Brantes-Glavic, S., 2018. Normal menstrual cycle. In: O. Lutsenko, ed., *Menstrual Cycle*. London: IntechOpen.

13. Reed, B.G. and Carr, B.R. (2018). The Normal Menstrual Cycle and the Control of Ovulation. [online] www.ncbi.nlm.nih.gov. MDText.com, Inc. Available at: https://www.ncbi.nlm.nih.gov/books/NBK279054/?report=printable

14. **Sa**hah, P., Kumar, R., Singh, A. and Patel, M., 2021**.** Regulation of FSH levels and Sertoli cell function in male reproduction. *Reproductive Biology*, 21(1):12–19.

15. Thiyagarajan, D.K., Basit, H. and Jeanmonod, R. (2019). Physiology, menstrual cycle. [online] National library of medicine. Available at: https://www.ncbi.nlm.nih.gov/books/NBK500020/

16. Wang, N., Shao, H., Chen, Y., Xia, F., Chi, C., Li, Q., Han, B., Teng, Y., and Lu, Y. (2017) 'Follicle-stimulating hormone, its association with cardiometabolic risk factors, and 10-year risk of cardiovascular disease in postmenopausal women', Journal of the American Heart Association, 6(9), e005918. doi: 10.1161/JAHA.117.005918.

17. Zolotnik, M.G. (2016). Endocrine-Disrupting Chemicals and Reproductive Health. *Journal of Midwifery & Women's Health*; *61*(4) :442–455.

Ethical Approval

Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medical Laboratory Science, Nnamdi Azikiwe University (Reference No.: FMLS/REC/025/075). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki of the World Medical Association. All participants' information was handled with strict confidentiality throughout the study.