

**EVALUATION OF THE EFFECTS OF 3,4-
METHYLENEDIOXYMETHAMPHETAMINE (MDMA) ON FEMALE
REPRODUCTION IN ADULT WISTAR RATS**

BY

**EFERAVWARE, Aghogho Success
(PG/BMS1817784)**

**DEPARTMENT OF ANATOMY,
COLLEGE OF MEDICAL SCIENCES,
UNIVERSITY OF BENIN,
BENIN CITY, EDO STATE.**

SEPTEMBER, 2025

**EVALUATION OF THE EFFECTS OF 3,4-
METHYLENEDIOXYMETHAMPHETAMINE (MDMA) ON FEMALE
REPRODUCTION IN ADULT WISTAR RATS**

BY

**EFERAVWARE, Aghogho Success
(PG/BMS 1817784)**

**A THESIS WRITTEN IN THE DEPARTMENT OF ANATOMY,
SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES,
AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF
DOCTOR OF PHILOSOPHY (Ph.D.) IN ANATOMY OF THE
UNIVERSITY OF BENIN**

SEPTEMBER, 2025

DECLARATION

I, Aghogho Success Eferavware with Matriculation Number: PG/1817784, hereby declare that this dissertation titled: "Evaluation of the Effects of 3,4-Methylenedioxymethamphetamine (MDMA) on Female Reproduction in Adult Wistar Rats" was carried out by me in the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin, under the supervision of Professor Christopher Lucky Sakpa and Dr. Chukwuma Vitalis Ezeoku.

This research is my original work and has not been submitted in part or whole for the award of any degree or diploma in this or any other institution of higher learning. All sources of data and scholarly contributions have been duly acknowledged in accordance with academic standards.

I take full responsibility for the integrity and content of this dissertation.

Aghogho Success Eferavware

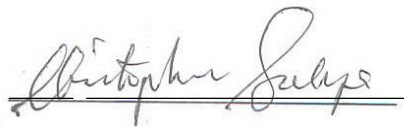
Date: _____

PLAGIARISM CERTIFICATION

We, the undersigned, hereby certify that the dissertation titled: "Evaluation of the Effects of 3,4-Methylenedioxymethamphetamine (MDMA) on Female Reproduction in Adult Wistar Rats" submitted by Aghogho Success Eferavware, Matriculation Number: PG/BMS1817784, of the Department of Anatomy, is an original work carried out by the candidate under our supervision. To the best of our knowledge, this work is free from plagiarism and contains no material previously published or written by another person, except where duly cited. It has not been submitted, either in whole or in part, for the award of any other degree or diploma in this or any other institution. This research has been subjected to plagiarism detection using approved software, and the report is available on request. We affirm that proper academic and ethical standards were maintained throughout the research process.

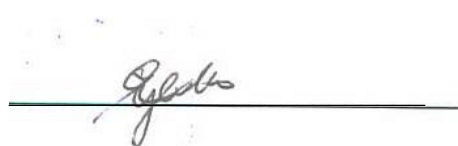
Aghogho Success Eferavware

Candidate



Professor Christopher Sakpa

Principal Supervisor



Dr. Chukwuma Vitalis Ezeuko

Co-Supervisor

Date: _____

Date: 29/09/25

Date: 29/09/25

AUTHOR'S STATEMENT

I hereby grant the University of Benin, through the University of Benin Library, a non-exclusive, worldwide right to reproduce and distribute my thesis and abstract (hereinafter "the Work"), in whole or in part, through any media, in its present form or any translated version for preservation and accessibility, provided such translation does not alter its content. This grant is royalty-free, and I retain the right to publish the Work in its current or future versions elsewhere.

Warranties

I further affirm that:

1. I am the sole author of the Work and grant the University of Benin the right to make it available four (4) years after the award of my doctorate degree, in compliance with the University of Benin Senate regulations.
2. The Work does not contain confidential information requiring third-party consent for disclosure.
3. I have exercised due diligence to ensure that the Work is original and does not breach any Nigerian law or infringe upon any third party's copyright or other Intellectual Property Rights, to the best of my knowledge.
4. Where the Work includes copyrighted material not owned by me, I have obtained unrestricted permission from the copyright holder to grant this license to the University of Benin Library. Such third-party materials are clearly identified and acknowledged within the Work.
5. In the event of any copyright dispute concerning the Work, I agree to indemnify and hold harmless the University of Benin, its officers, employees, and agents from any liability arising from the material authorized under this agreement.
6. The University of Benin is under no obligation whatsoever to take legal action on my behalf as the Depositor in the event of an intellectual property rights infringement or any other related dispute in the material deposited.

Author's Name

Christopher Julius

Signature/Date

29/09/25

Email

Supervisor's Name

[Signature]

Signature/Date

29/09/25

Email

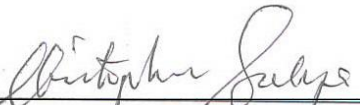
Supervisor's Name

Signature/Date

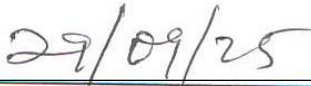
Email

CERTIFICATION


We certify that this research work was carried out by Aghogho Success Eferavware, with Matriculation Number: PG/BMS/1817784, in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. In partial fulfilment of the award of Ph.D in Anatomy under the supervision of: Prof. C.L. Sakpa and Dr. V.C. Ezeuko. All literatures and other sources of information consulted, quoted or used in this research have been acknowledged and properly referenced.




Prof. C.L. Sakpa (Supervisor)



Date



Dr. V.C. Ezeuko (Co-Supervisor)



Date

Dr. A.B. Enogieru
Head of Department

Date

External Examiner

Date

DEDICATION

This work is lovingly dedicated to **God Almighty**, the One who authored this journey and saw me through every chapter. To Him be all the glory.

ACKNOWLEDGEMENT

First and foremost, I give all glory and thanks to God Almighty, the source of my wisdom, strength, resilience, and good health throughout this PhD journey. Without His grace, this accomplishment would have remained impossible.

My deepest gratitude goes to my main supervisor, Professor Christopher Sakpa, for his invaluable guidance, patience, and mentorship. Your constructive critiques and encouragement shaped this research and refined my academic journey.

I sincerely appreciate my co-supervisor, Dr. Vitalis, for his critical insights, support, and encouragement, which greatly enriched this study.

Special thanks go to Professor Lilian Ozoko, my BSc and MSc supervisor, for laying a solid academic foundation, and to Professor Essy Issah, my MPH supervisor, for broadening my vision in public health.

I acknowledge with heartfelt gratitude the entire staff of the Department of Anatomy, University of Benin, including the professors, for their leadership and inspiration. The Lecturers, for their dedication, teaching, and mentorship throughout my academic growth. The Non-academic staff, for their administrative, technical, and moral support that created a conducive environment for my studies.

Special appreciation also goes to the laboratory assistant, for the commitment and professionalism during my experiments, and to my fellow postgraduate students, for their constant readiness to help.

I am grateful to Benson Idahosa University, for awarding me a research grant, and to the University of Benin, for providing the enabling environment, resources, and facilities that supported the success of this work. "

TABLE OF CONTENTS

TITLE PAGE -----	ii
DECLARATION -----	iii
CERTIFICATION OF THESIS ON PLAGIARISM -----	iv
CERTIFICATION -----	v
DEDICATION -----	vi
ACKNOWLEDGEMENTS -----	vii
TABLE OF CONTENTS -----	ix
LIST OF TABLES -----	xiv
LIST OF FIGURES -----	xv
LIST OF PLATES -----	xvi
LIST OF ABBREVIATIONS -----	xvii
ABSTRACT -----	xxiii

CHAPTER ONE

INTRODUCTION

1.1	Background of the Study -----	1
1.2	Statement of Research Problem -----	4
1.3	Significance of the Study -----	5
1.4	Scope of the Study -----	6
1.5	Research Questions -----	7
1.6	Objective of the Study -----	8
1.6.1	General Objective -----	8
1.6.2	Specific Objectives -----	8

CHAPTER TWO

LITERATURE REVIEW

2.1.	Drug of the Study -----	9
2.2	Discovery of MDMA -----	10

2.2.1	Early Research and Applications -----	10
2.2.2	Rising Recreational Use -----	12
2.2.3	History of Substance Use in Africa: From Transit Routes to Illicit Drug Markets---	13
2.3	Society and Culture -----	16
2.3.1	Legal Status of MDMA -----	16
2.4	Government Initiatives and Institutional Responses to Drug Abuse in Nigeria and Beyond -----	17
2.5	Pharmacodynamics of MDMA-----	23
2.5.1	Pharmacokinetics of MDMA -----	23
2.6	Impact on Neuroendocrine Regulation-----	26
2.7	Chemistry -----	26
2.8	Potential Use in Medicine -----	27
2.9	MDMA Toxicity-----	28
2.9.1	Effects on the User-----	28
2.9.2	Undesired Effects-----	29
2.9.3	Long-Term or Residual Effects-----	29
2.9.4	Long-Term Psychiatric Effects-----	30
2.9.5	Physical Complications-----	31
2.9.6	Severe Physical Toxicity-----	31
2.9.7	Fatalities Linked to Ecstasy -----	33
2.9.8	Addiction -----	34
2.9.9	Comparison with Other Drugs -----	35
2.10	Reproduction-----	36
2.10.1	Reproductive Problems in General-----	37
2.10.2	Problems Associated with Female Reproduction-----	37
2.10.3	Pathogenic Mechanisms in Reproductive Disorders Affecting Pregnancy Outcomes -----	39
2.11	Infertility-----	40
2.12	Animal Model Utilized in the Study-----	41

2.12.1	Animal of the Study-----	41
2.12.5	Morphological Features of the Vagina, Uterus, and Ovaries Across the Four Estrous Phases in a Normally Cycling Adult Rat-----	43
2.12.6	Mating and Reproductive Behavior-----	47
2.12.7	Pregnancy Detection in Wistar Rats-----	47
2.12.8	Recognition of Pregnancy in Wistar Rats-----	48
2.12.9	Embryo Implantation in Wistar Rats-----	49
2.12.10	Gestation, Parturition, and Weaning-----	50
2.13	Organs of the Study-----	51
2.13.1	Ovary of Wistar Rat-----	51
2.13.2	Ovarian Morphology and Folliculogenesis in Wistar Rats-----	52
2.13.3	Uterus of Wistar Rats-----	54
2.13.4	Placenta of the Wistar Rat-----	55
2.13.5	The Pituitary Gland of the Wistar Rat-----	57
2.14	Regulation of Anterior Pituitary Hormone Secretion-----	60
2.14.1	Development of the Pituitary Gland-----	62
2.14.2	Blood Supply, Lymphatics, and the Hypophyseal Portal System-----	63
2.15	Female Fertility Hormones-----	64
2.15.1	Gonadotrophic Hormones-----	64
2.15.2	Prolactin-----	66
2.15.3	Estrogen-----	68
2.15.4	Progesterone-----	72
2.15.5	Testosterone-----	74
2.16	Gametogenesis-----	78
2.16.2	Meiosis-----	80
2.16.3	Hormonal Regulation of Oogenesis-----	83
2.16.4	Steroid Hormone Function in Oogenesis-----	84
2.17	Effects of MDMA-----	87
2.17.1	Effects on Female Reproductive Health-----	87

2.17.2	Effects on Female Reproductive Function-----	88
2.17.3	Embryotoxicity and Fetal Development-----	89
2.17.3	Embryotoxic and Neurodevelopmental Outcomes-----	89
2.17.4	Reproductive and Developmental Toxicity in Mice-----	90

CHAPTER THREE

MATERIALS AND METHODS

3.1.	Ethics Approval-----	91
3.2.	Materials-----	91
3.2.1	Hormone Assay Kit-----	91
3.2.2	Equipment-----	92
3.2.3	Softwares-----	93
3.2.4	Drug-----	93
3.2.5	Drug Acquisition -----	93
3.2.6	Acute Toxicity Study-----	93
3.2.7	Experimental Animals and Categorization-----	94
3.2.8	Experimental Design-----	95
3.2.9	Mating Protocol-----	97
3.2.10	Administration of Drug-----	97
3.2.11	Monitoring of Rats-----	98
3.2.12	Methods-----	98
3.2.13	Sacrifice and Tissue Collection-----	100
3.3	Hormone Analysis-----	103
3.3.2	Anti-oxidant Procedure-----	104
3.4	Histological Procedure-----	108
3.5	Data Analysis-----	110

CHAPTER FOUR

RESULTS

4.1 Pregestational Study----- 111
4.2 Pre-gestation Photomicrographs----- 124
4.3 Gestational Study----- 144

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion----- 152
5.2 Conclusion----- 167
5.3 Summary of Findings----- 167
5.4 Recommendations----- 168
5.5 Contribution to Knowledge----- 169
5.6 Limitation of the Study----- 170

REFERENCES----- 172

APPENDIX----- 211

ETHICAL APPROVAL LETTER----- 211

NDLEA APPROVAL----- 212

LIST OF TABLES

Table 2.1: Controlled Substances Act Classifications-----	18
Table 2.2: DEA’s Eight-Factor Drug Scheduling Analysis-----	20
Table 2.3: The DEA’S “New” Five Part Test-----	22
Table 4.1.1: Somatic and Reproductive Organ Weight Indices in Female Wistar Rats-----	119
Table 4.1.2a: Hormone Analysis Outcomes for Pre-gestational Study-----	120
Table 4.1.2b: Estradiol/Testosterone ratio showing dose-dependent hormonal imbalance across MDMA treatment groups-----	120
Table 4.1.3: Pregnancy Success Rate and Outcomes at pre-gestation stage-----	121
Table 4.1.4: Oxidative Study of the Ovary-----	122
Table 4.1.5: Oxidative Study of the Uterus-----	123
Table 4.3.1: Pregnancy Outcomes at Germinal Phase of Dams Dissected at GD 15-----	147
Table 4.3.2: Pregnancy Outcomes at Embryonic Phase of Dams Dissected at GD20-----	148
Table 4.3.3: Pregnancy Outcomes at Embryonic Phase of Dams Littered at GD21-----	149
Table 4.3.4: Pregnancy Outcomes at Fetal Phase of Dams Sacrificed at GD21-----	150
Table 4.3.5: Pregnancy Outcomes at Fetal Phase of Dams Littered at GD21-----	151

LIST OF FIGURES

Figure 2.1: Phase I and phase II metabolism of MDMA-----	25
Figure 2.2: Median sagittal view of the human brain illustrating the anatomical relationship between the hypothalamus, pituitary gland, and pineal gland-----	61
Figure 2.3: Anatomical organization of the hypothalamus and pituitary gland-----	62

LIST OF PLATES

Plate 4.2.1a: Adenohiphysis from control group (H&E; 400x)-----	124
Plate 4.2.1b: Adenohiphysis from 80mg/kg group (H&E; 400x)-----	125
Plate 4.2.1c: Adenohiphysis from 160mg/kg group (H&E; 400x)-----	126
Plate 4.2.2a: Ovary Control (H&E; 40x) (Scale bar = 400 μm.)-----	127
Plate 4.2.2b: Ovary Control (H&E; 100x)-----	128
Plate 4.2.3a: Ovary from 80mg group (H&E; 40x)-----	129
Plate 4.2.3b: Ovary from 80mg group (H&E; 100X)-----	130
Plate 4.2.3c: Ovary from 80mg group (H&E; 400X)-----	131
Plate 4.2.4a: Ovary from 160mg group (H&E; 40x)-----	132
Plate 4.2.4b: Ovary from 160mg group (H&E; 100x)-----	133
Plate 4.2.4c: Ovary from 160mg group (H&E; 400x)-----	134
Plate 4.2.5a: Uterus from control group (H&E; 40x)-----	135
Plate 4.2.5b: Endometrium from control group (H&E; 400x)-----	136
Plate 4.2.5c: Myometrium from control group (H&E; 400x)-----	137
Plate 4.2.6a: Uterus from 80mg/kg group (H&E; 40x)-----	138
Plate 4.2.6b: Endometrium from 80mg/kg group (H&E; 400x)-----	139
Plate 4.2.6c: Myometrium from 80mg/kg group (H&E; 400x)-----	140
Plate 4.7a: Uterus from 160mg/kg group (H&E; 40x)-----	141
Plate 4.7b: Endometrium from 160mg/kg group (H&E; 400x)-----	142
Plate 4.7c: Myometrium from 160mg/kg group (H&E; 400x)-----	143

LIST OF ABBREVIATIONS

5-HT: Serotonin

AC: Acidophils

ACTH: Adrenocorticotropic Hormone

ADH: Antidiuretic Hormone

AEND: Atrophic Endometrium

ALJ: Administrative Law Judge

ANOVA: Analysis of Variance

ART: Assisted Reproductive Technologies

BA: Basophils

Bax: BCL2-Associated X protein

Bcl-2: B-cell lymphoma 2

BPA: Bisphenol A

BZ: Basal Zone (of the placenta)

CAT: Catalase

CH: Chromophobes

CIF: Cellular Infiltrate

CL: Corpus Luteum

CNS: Central Nervous System

COMT: Catechol-O-Methyltransferase

CRL: Crown-Rump Length

CSF: Cerebrospinal Fluid

CV: Cytoplasmic Vacuolation

CYP2D6: Cytochrome P450 2D6

CYP3A4: Cytochrome P450 3A4

DA: Dopamine

DB: Decidua Basalis

DF: Degenerating Follicles

DIC: Disseminated Intravascular Coagulation

DMF: Degenerated Muscle Fibers

E2: Estradiol

E2/T Ratio: Estradiol to Testosterone Ratio

ECG: Electrocardiogram

EDCs: Endocrine-Disrupting Chemicals

EDTA: Ethylenediaminetetraacetic Acid

EG: Endometrial Glands

ELISA: Enzyme-Linked Immunosorbent Assay

EMCDDA: European Monitoring Centre for Drugs and Drug Addiction

ER α : Estrogen Receptor alpha

ER β : Estrogen Receptor beta

ET: Endometrial Thinning

FDA: Food and Drug Administration (U.S.)

FGR: Fetal Growth Restriction

FSH: Follicle-Stimulating Hormone

GABA: Gamma-Aminobutyric Acid

GA: Glandular Atrophy

GDM: Gestational Diabetes

GD: Gestational Day

GH: Growth Hormone

GnRH: Gonadotropin-Releasing Hormone

GPx: Glutathione Peroxidase

H&E: Haematoxylin and Eosin

H₂O₂: Hydrogen Peroxide

HCl: Hydrochloric Acid

HC: Hyperchromasia

HHS: Health and Human Services (U.S. Department of)

HMMA: 4-Hydroxy-3-Methoxymethamphetamine (MDMA metabolite)

HPG axis: Hypothalamic-Pituitary-Gonadal axis

HRT: Hormone Replacement Therapy

HV: Hypophyseal Vessels

ICM: Inner Cell Mass

ICSH: Interstitial Cell-Stimulating Hormone

IL-6: Interleukin-6

IND: Investigational New Drug

IUGR: Intrauterine Growth Restriction

IUPAC: International Union of Pure and Applied Chemistry

KMnO₄: Potassium Permanganate

LD₅₀: Median Lethal Dose

LES: Loose Edematous Stroma

LH: Luteinizing Hormone

LIF: Lymphocytic Infiltration

LOAEL: Lowest Observed Adverse Effect Level

LSD: Lysergic Acid Diethylamide

LZ: Labyrinth Zone (of the placenta)

MDA: 3,4-Methylenedioxyamphetamine (MDMA metabolite) / Malondialdehyde

MDEA: 3,4-Methylenedioxy-N-ethylamphetamine

MDMA: 3,4-Methylenedioxymethamphetamine

MF: Maturing Follicles

MG: Metrial Gland

MII: Metaphase II (stage of oocyte maturation)

MSH: Melanocyte-Stimulating Hormone

Na₂CO₃: Sodium Carbonate

NaHCO₃: Sodium Bicarbonate

NaCl: Sodium Chloride

NAFDAC: National Agency for Food and Drug Administration and Control (Nigeria)

NDA: New Drug Application

NDLEA: National Drug Law Enforcement Agency (Nigeria)

NE: Norepinephrine

NIDA: National Institute on Drug Abuse (U.S.)

NOAEL: No Observed Adverse Effect Level

OD: Optical Density

OSE: Ovarian Surface Epithelium

PCN: Pharmacists Council of Nigeria

PCOS: Polycystic Ovary Syndrome

PD: Pars Distalis (of the pituitary gland)

PFAS: Per- and Polyfluoroalkyl Substances

PI: Pars Intermedia (of the pituitary gland)

PIBF: Progesterone-Induced Blocking Factor

PIF: Prolactin-Inhibiting Factor

PMK: Piperonyl Methyl Ketone (MDMA precursor)

PMA: Paramethoxyamphetamine

PN: Pars Nervosa (of the pituitary gland)

POMC: Pro-opiomelanocortin

PR: Progesterone Receptor

PRF: Prolactin-Releasing Factor

PRL: Prolactin

PT: Pars Tuberalis (of the pituitary gland)

PTB: Preterm Birth

PYK: Pyknotic Nuclei

ROS: Reactive Oxygen Species

SC: Sinusoid Congestion

SD: Stroma Dilatation

SDGs: Sustainable Development Goals

SEM: Standard Error of the Mean

SI: Sinusoids

SMD: Smooth Muscle Distortion

SOD: Superoxide Dismutase

SPECT: Single-Photon Emission Computed Tomography

SPSS: Statistical Package for the Social Sciences

STC: Stroma Congestion

SV: Stroma Vacuolation

TAAR1: Trace Amine-Associated Receptor 1

TBA: Thiobarbituric Acid

TCA: Trichloroacetic Acid

TNF- α : Tumor Necrosis Factor-alpha

U.S. DEA: United States Drug Enforcement Administration

UNODC: United Nations Office on Drugs and Crime

uNK cells: Uterine Natural Killer cells

VC: Vascular Congestion

VD: Vasodilation

VMAT2: Vesicular Monoamine Transporter 2

VST: Vascularized Stroma

β -LPH: Beta-Lipotropin

ABSTRACT

The increasing recreational use of 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") among women of reproductive age raises critical concerns regarding fertility and pregnancy outcomes. This study investigated the reproductive and developmental toxicity of MDMA in adult female Wistar rats, focusing on hormonal regulation, oxidative stress, histopathology, and gestational effects following pre-gestational and gestational exposure.

A total of 140 Wistar rats (120 females, 20 males) were assigned to pre-gestational (Category A) and gestational (Category B) protocols. Treated groups received oral MDMA at 80 mg/kg and 160 mg/kg, while controls received distilled water. Serum levels of FSH, LH, PRL, estradiol, progesterone, and testosterone were measured alongside oxidative stress markers (SOD, CAT, GPx, MDA) and histological analyses of the pituitary, ovaries, and uterus. Data were analyzed using one-way ANOVA with LSD post hoc test ($p < 0.05$).

MDMA induced dose-dependent reductions in body and reproductive organ weights, likely due to serotonergic suppression of appetite, oxidative stress, and mitochondrial dysfunction. Hormonal assays revealed significant disruption of the hypothalamic-pituitary-gonadal (HPG) axis, including reduced LH ($p = 0.02$), elevated estradiol ($p = 0.00$), and progesterone ($p = 0.05$). A biphasic testosterone response was observed: reduced in the 80 mg/kg group (0.48 ± 0.00 ng/mL vs. 1.12 ± 0.14 ng/mL in controls) and elevated in the 160 mg/kg group (1.48 ± 0.09 ng/mL; $p = 0.00$), suggesting dysregulation of androgen synthesis via theca cell dysfunction or disrupted HPG feedback. Reproductive outcomes mirrored endocrine alterations. Pre-gestational exposure reduced conception rates (100% in controls vs. 20% and 0% in treated groups). Gestational exposure impaired implantation, fetal viability, and growth, leading to increased resorptions, intrauterine growth restriction, stillbirths, and postnatal abnormalities. Biochemical assays revealed dose-dependent suppression of antioxidant enzymes and altered lipid peroxidation, indicating oxidative damage in ovarian and uterine tissues.

Histopathological evaluation showed progressive degeneration: the pituitary exhibited chromophobe predominance and vacuolation; ovaries showed follicular atresia, degeneration, and vascular injury; and uterine tissues demonstrated glandular atrophy, edema, inflammation, and myometrial disruption. These structural changes aligned with the observed biochemical and hormonal abnormalities.

In conclusion, MDMA exposure before or during pregnancy disrupts female reproductive function in a dose-dependent manner. It impairs fertility, alters endocrine signaling, induces oxidative stress, and causes tissue-specific toxicity, with profound consequences for implantation and fetal development. These findings reinforce the public health risks of MDMA use during reproductive years and the need for targeted reproductive toxicology awareness.

Keywords: MDMA, female reproduction, Wistar rats, endocrine disruption, oxidative stress, fertility, histopathology, pregnancy outcome.