EFFECTS OF TENOFOVIR DISOPROXIL FUMURATE/LAMIVUDINE/ DOLUTEGRAVIR (TLD) ON THE HISTOMORPHOMETRIC AND REPRODUCTIVE PARAMETERS OF THE OVARY, UTERUS, AND PLACENTA OF ADULT WISTAR RATS

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SEPTEMBER, 2025

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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 declare that:

This thesis report is based on the study undertaken by me in the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria, under the supervision of Prof. J.E Ataman (Project Supervisor) and Dr. V.C Ezeuko (Project Co- Supervisor).

This work has not been previously submitted for the award of a degree.

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DEDICATION

First and foremost, I dedicate this thesis to God Almighty for His guidance, provision, and mercy that have sustained me throughout this transformative journey. Secondly, I dedicate it to my beloved mummy, Mrs. Ogbe Grace, and my late daddy, Elder Ogbe Gabriel Edafe, whose unwavering support and passion for my education have been a constant source of strength. God bless you.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Prof. J. E. Ataman for being an exceptional supervisor and making this thesis journey a truly rewarding experience. You will always remain a significant part of my success story. Your unwavering support, insightful guidance on experimental techniques and data analysis, which you patiently helped me to understand, as well as your genuine interest in this study, have been instrumental in shaping the outcome of this thesis. Your brilliant ideas, expert advice, and dedication in reviewing multiple drafts of this thesis are deeply appreciated. Your constant encouragement to strive for excellence has motivated me to work harder and see this program through to completion.

I am also sincerely thankful to my co-supervisor, Dr. Vitalis Chukwuma Ezeuko, for his valuable insights, contributions, and for generously sharing his knowledge and experience in experimental research. May God bless you all abundantly.

I would like to extend my sincere appreciation to the Head of Department, Dr. A. B. Enogieru, as well as all academic and non-academic staff of the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences. Your collective support played a vital role in the successful completion of this thesis. Thank you, and may God bless you all.

I am particularly grateful to Nweke Samuel Monday and Raymond Joseph Enoghase (ALIVE Practical Study) for their assistance during my bench work in the laboratory. Your guidance and insights greatly enhanced the quality of my research - thank you.

My heartfelt thanks go to Mr. Valentine Igbafe for his prayers and words of encouragement, which constantly motivated me to give my best. Your support has meant so much to me. May God continue to bless you.

Special thanks to Mr. Omogbai Wisdom Ohiwerei for your invaluable advice on data analysis and for your continued encouragement and insight - you made this journey significantly smoother and more fulfilling. To the entire staff of Ohilux Global Laboratory and Training Institute, Ekpoma, Edo State - I deeply appreciate your support and contributions. Thank you, and may God bless you all.

I have also been incredibly fortunate to receive unwavering support from friends, especially Dr. Michael Anozie Amadi, Dr. Onyilo Peace Omewomano, Dr. Edem Gabriel Donatus, Dr. Olugbenga Mary Adeola, Mr. Newtons Osarumwense Osagiede, Dr. Akpamu Uwuifor, Dr. Edebiri Ogbemudia Endurance, Dr. Willy Barinem Vidona, and Mr. Blackie Okosun Hassan. Thank you all for your love, friendship, and concern, which kept me grounded and sane throughout this journey.

To my friends who stood by me during the most stressful times - Mr. Okolosi Patani Innocent Edewor, Mrs. Gbobo Dorcas Daunakige Allagoa, Mrs. Godspower Tarikebi, Mrs. Excel Nneka Herrita, Dr. Yembra Aghogho Tarma, and Engr. (Mrs). Uwen John Uduak - thank you for your encouragement and unwavering support in countless ways. May God bless you and your families.

I also extend my deepest appreciation to my colleagues at my workplace, past and present: Dr. William Abiehode, Mr. Sixtus Ojinnaka, Dr. Golda Ezeh, Mrs. Eyaal Alali, Mrs. Okon Peace, Osuo-Odaremete Epow-Aswei Solomon Serene, Ogolo Rubby Abinye, and Emeka Orlu Adna Eze Esther. Your cooperation, encouragement, and patience throughout this journey have been invaluable. Without your support, I could not have completed this Ph.D. successfully.

I remain eternally indebted to Mrs. Oghenegweke Odoh Jessica, Igara Caleb, Igara Abigail, Oghenevwairhe Orunuaherhe, and Orunuaherhe Rosemary. You welcomed a complete stranger into your homes and hearts in Edo State during a time of urgent need, making me feel like family. Your generosity and kindness will never be forgotten.

To Pst. and Pst. (Mrs.) Hope Kalio, Pst. Peter Abadi, Pst. Joshua Dabibi, Pst. and Dr. (Mrs.) Darlington Emeka, and all members of Greater Evangelism World Crusade Sand Filled Church 1, Glorious Centre U.P.E Sand Filled Church 1 (20 Rooms Area), Borokiri, Port Harcourt, Rivers State - thank you for your unwavering prayers throughout my Ph.D. program.

My deepest gratitude goes to my beloved parents, Mr. and Mrs. Ogbe Gabriel Edafe. You have been the foundation of my strength and confidence. Your moral, financial, and emotional support - along with your unwavering belief in me - have shaped me into the woman I am today. Though my father is no longer physically present, I still feel his prayers, support, and guidance. Daddy, your words continue to inspire and anchor me. I pray that you rest in the bosom of our Lord Jesus Christ. Mummy, this is your success too - may you live long in health and happiness to witness many more milestones. Words cannot fully express my love and appreciation for you.

Lastly, I want to express heartfelt thanks to my family: Sis. Ogbe Avoezereye Deborah, Mr. and Mrs. Igara, Mr. and Mrs. Ajiroghene Jacob, Enejeta Enifome Julius, Mr. and Mrs. Okoh, Ogbe Omoviagho Elisha, Ogbe Joshua Akpoghene and Mr. and Mrs. Oghenekparobo Enejeta Godwin. Thank you for never doubting my abilities, even when I did. Your constant love, encouragement, and belief in me gave me the strength to push through. May God bless you all. I love you deeply.

This thesis has truly been a collaborative effort, and I am profoundly grateful to everyone who contributed to its realization. Thank you all from the bottom of my heart.

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ABBREVIATION

17β-HSD - 17β-Hydroxysteroid Dehydrogenase

3TC – Lamivudine

3TC-MP or L-MP – Lamivudine Mono Phosphate

3TC-TP or L-TP - Lamivudine Triphosphate

3β-HSD - 3β - Hydroxy Steroid Dehydrogenase

A – Androstenedione

ACTH - Adenocorticoid Hormone

AF - Atretic Follicle

AIDS - Acquired Immunodeficiency Syndrome

AMH - Anti-Müllerian hormone

ANOVA - Analysis of Variance

API - Active Pharmaceutical Ingredient

APR - Antiretroviral Pregnancy Registry

ART - Antiretroviral Therapy

ART - Assisted Reproductive Technology

ARVs – Antiretroviral

ATP - Adenine Triphosphate

ATV – Atazanavir

AZT – Zidovudine

BDH - British Drug House

BV - Blood vessel

C – Control

$C_{10}H_{14}N_2O_8$ - Ethylenediaminetetraacetic Acid (EDTA)

$C_2H_3O_2CL$ - Trichloroacetic Acid

CA - Corpus Albicans

cART - Combination Antiretroviral Therapy

CAT – Catalase

CD4+ cells - Cluster of Differentiation Antigen 4

CDC – Centres for Disease Control and Prevention

CHMP - Committee for Medicinal Products for Human Use

CI - Confidence Interval

CL - Corpus Luteum

CRL - Crown-Rump Length

CY - Cytotrophoblast

CYP - Carbamoyl Pyridone

D - Decidua Basalis

Da – Daltons

Dcl - Degenerating Corpus Luteum

dFBV - Dilated Fetal Blood Vessel

DHEA – Dehydroepiandrosterone

DHEAS - Dehydroepiandrosterone Sulphate

DHT – Dihydrotestosterone

dL - Deciliter

dMBV - Dilated Maternal Blood Vessel

DNA - Deoxyribonucleic Acid

DPX - Dibutylphthalate Polystyrene Xylene

DR Congo - Democratic Republic of Congo

DTG – Dolutegravir

dT4 – Stavudine

DTNB - 5, 5-dithio-bis-2-nitrobenzoic acid

dV - Dilated Vacoulation

E – Endometrium

E1 – Estrone

E2 – Estrogen

E2 – Estradiol

E3 – Estriol

EC - Endometrial Canal

EACS - European Aids Clinical Society

ECM - Extracellular Matrix

EDTA - Ethylenediaminetetraacetic Acid

EE - Exogenous Estrogen

EFV – Efavirenz

EG - Endometrial Glands

EGF - Epidermal Growth Factors

ELISA - Enzyme-Linked Immunosorbent Assay

EMA - European Medicine Agency

ENTs - Equilibrate Nucleoside Transporters

Ep - Columnar Epithelium

ERs - Estrogen Receptors

ETR – Etravirine

FA - Follicular Antrum

FB - Fetal Blood

FBV - Fetal Blood Vessel

FC - Follicular Cysts

FDA - United State Food and Drug Administration

FDC - Fixed Dose Combination

FMOH - Federal Ministry of Health

FSH - Follicle Stimulating Hormone

g – Gram

GCI - Glycogen Cell Island

GD - Gestation Day

GF - Graffian Follicle

GnRH - Gonadotropin-Releasing Hormone

GPx - Glutathione Peroxidase

GSH - Reduced Glutathione

H&E - Haematoxylin and Eosin

H_2O_2 - Hydrogen Peroxide

H_2SO_4 - Tetraoxosulphate Acid (VI)

HAART - Highly Active Antiretroviral Therapy

HB-EGF - Heparin Binding-Epidermal Growth Factors

hCG - Human Chorionic Gonadotropin

HCl - Hydrochloric Acid

HIV - Human Immunodeficiency Virus

HLD - Height of Luminal Diameter

hOAT1 - Human Organic Anion Transporters

HPG - Hypothalamus-Pituitary-Gonadal Axis

HUD - Height of Luminal Diameter

ICM - Inner Cell Mass

IFN – Interferon

INSTI - Integrase Strand-Transfer Inhibitors

IUGR - Intrauterine Growth Restriction

IVC - Inferior Venacava

IVF - In-vitro Fertilization

$KMnO_4$ - Potassium Permanganate

KOH - Potassium Hydroxide

LD50 - Lethal Dose

LH - Luteinizing Hormone

LIF - Leukamea Inhibitory Factors

LMIC - Low and Middle Income Countries

LPV/r - Lopinavir/Ritonavir

LSD - Least Significant Difference

Ltd – Limited

M – Myometrium

MC4R genes - Melanocortin 4 Receptor Genes

MDA – Malondialdehyde

MDR - Multidrug Resistance

MDR4 - Multiple Drugs Resistant

Ml – Millilitre

mm – Millimetre

MMP-9 - Matrix Metalloproteinase-9

MS - Maternal Sinusoids

MTCT - Mother-to-Child Transmission

mtDNA - Mitochondrial DNA

MUC-1 - Mucin Like Integral Membrane Protein

Na_2CO_3 - Sodium Carbonate

Na_2HPO_4 - Disodium Phosphate

$Na_3C_6H_5O_7$ - Sodium Citrate

NACA - National Agency for the Controls of AIDs

NaCl - Sodium Chloride

NaH_2PO_4 - Sodium Dihydrogen Phosphate

$NaHCO_3$ - Sodium Hydrogen Carbonate

NaH PO_4 - Sodium Hydrogen Phosphate

NaOH - Sodium Hydroxide

NASCP - National AIDS and STIs Control Programme

NFV – Nelfinavir

NIH - National Institutes of Health

nm - Nanometer

NNRTIs - Non-Nucleoside Reverse Transcriptase Inhibitors

NRTIs - Nucleoside Reverse Transcriptase Inhibitors

NTD - Neural Tube Defects

O^2 - Superoxide Anions

OCT2 - Organic Cation Transporter 2

oLC - Occluded Uterine Luminal Canal

OS - Oxidative stress

OS - Ovarian Stroma

OSE - Ovarian Surface Epithelium

P – Perimetrium

P4 – Progesterone

P450 - Cytochrome P450 Protein

PCNA - Proliferating Cell Nuclear Antigen

PCOS - Polycystic Ovary Syndrome

PCP - Pneumocystis Pneumonia

PEP - Post Exposure Prophylaxis

pH - Phosphate Buffer

PI - Protease Inhibitor

PL - Placenta Labyrinth

PL - Human Placenta Lactogen

PLHIV - People Living with HIV

PMS - Premenstrual Syndrome

PMTCT-Prevent the Mother-to-Child Transmission of HIV

PND - Postnatal Day

PR-A - Progesterone Receptor A

PR-B - Progesterone Receptor B

PRL – Prolactin

PW - Placental Weight

RAAS - Renal Renin Angiotensin Aldosterone System

Redox Status - GSH/GSSG

RNA - Ribonucleic Acid

RNS - Reactive Nitrogen Species

ROS - Reactive Oxygen Species

SBP - Systolic Blood Pressure

SEM - Standard Error of Mean

SGA - Small for Gestational Age

SHBG - Sex Hormone-Binding Globulin

SIV - Simian Immunodeficiency Virus

SIVcpz - Simian Immunodeficiency Virus in Chimpanzee

SIVsmm -Simian Immunodeficiency Virus in Sooty Mangabey Monkeys

SOD - Superoxide Dismutase

SOF – Sofosbuvir

SPSS - Statistical Package for Social Sciences

SSEs - Satisfying Sexual Episode

ST – Spongiotrophoblast

StAR - Steroidogenic Acute Regulatory Protein

STI - Sexually Transmitted Infection

SY – Syncytiotrophoblast

T – Testosterone

T – Treated

TAF – Alafenamide

TAF/3TC - Tenofovir Alafenamide/Lamivudine

TB – Tuberculosis

TBA - Thiobarbituric Acid

TCA - Trichloroacetic Acid

TDF - Tenofovir Disoproxil Fumarate

TGC - Trophoblastic Giant Cell

TGF-α - α-Transforming Growth Factors

TLD – Tenofovir Disoproxil Fumarate/Lamivudine/ Dolutegravir

TLE - Tenofovir Disoproxil Fumarate/Lamivudine/ Efavirenz

TP - Total Protein

TSH - Thyroid Stimulating Hormone

µM – Micromolar

U - Umbilical Cord

UA - Uterine Arteries

UD - Uterine Diameter

UECs - Uterine Epithelial Cells

UK - United Kingdom

UNAIDS – Joint United Nations Programme on HIV/AIDS

UNFPA - United Nations Population Fund

USA - United States of America

VSGA - Very Small for Gestational Age

WHO - World Health Organization

WLD - Width of Luminal Diameter

WUD - Width of Uterine Diameter

ZLN - Zidovudine Lamivudine, Nevirapine

ABSTRACT

Human Immunodeficiency Virus (HIV) infection remains one of the leading causes of morbidity and mortality worldwide. Since the beginning of the epidemic, approximately ninety-one million four hundred thousand people have been infected with HIV, and about forty-four million one hundred thousand have died from AIDS-related illnesses. Globally, in 2024, forty million eight hundred thousand people were living with HIV, six hundred thirty thousand people died from AIDS-related illnesses, and one million three hundred thousand people were newly infected with HIV. Tenofovir Disoproxil Fumarate/ Lamivudine/Dolutegravir (TLD) is one of the therapeutic regimens used in the management of HIV infection. This study assessed the effects of TLD on the histomorphometric and reproductive parameters of the ovary, uterus, and placenta of adult Wistar rats. A total of fifty adult female Wistar rats, weighing between 140 g and 194 g, were used for the study. The rats were randomly assigned to control and treated groups, consisting of twenty-five rats, and were further subdivided into five subgroups of five rats each. Both groups received growers mash and distilled water; however, the treated group was administered a daily oral dose of a combination drug consisting of Tenofovir Disoproxil Fumarate (5 mg/kg body weight), Lamivudine (5 mg/kg body weight), and Dolutegravir (0.8 mg/kg body weight) via an orogastric tube. After ninety days of TLD administration, animals with regular four-day estrous cycles were weighed and mated. The animals were categorized into pregestational, gestational, and postnatal groups. The pregestational group was used to evaluate the effects of TLD on antioxidant status, hormonal profile, and histomorphometry of the ovary and uterus. The gestational group was used to assess implantation/resorption, and placenta parameters, while the postnatal group was used to evaluate litter size, litter weight, intrauterine and neonatal death, growth retardation, and congenital anomalies. This study reveals that TLD treatment resulted in a significant decrease in body weight ($p < 0.05$) but did not significantly affect ovarian, uterine weights and uterine horn length ($p > 0.05$). Ovarian antioxidant status showed no significant changes; however, the uterus exhibited reduced malondialdehyde (MDA) levels and increased superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities. The placenta showed significantly reduced glutathione (GSH) levels. Serum testosterone levels were significantly reduced ($p < 0.05$). No significant differences were observed in litter number, implantation sites, or number of placenta ($p > 0.05$). However, litter size and placenta weights were significantly reduced, with evidence of intrauterine growth retardation and low birth weight. Histological findings revealed impaired follicular maturation characterized by atretic follicles, follicular and luteal cysts, narrowing of the endometrial cavity, and thickened endometrium in the treated group. Additionally, dilation, congestion, and vacuolation of the feto-maternal vascular bed were observed. In conclusion, TLD exerted notable adverse effects on reproductive parameters in female Wistar rats, suggesting the need for caution in its use among women of reproductive age.