HISTOLOGICALTERATOGENIC EFFECTS OF PRENATAL EXPOSURE TO VARIED DOSES OF DEXAMETHASONE ON FETAL PANCREAS IN ALBINO RATS

Ndung'u C. Wangui[1], Kweri Joseph[2], Mwangi Ann[3], Malik Atanas[4],

Department of Human Anatomy, Jomo Kenyatta University of Agriculture and Technology. Nairobi,Kenya.

IJASR 2021
VOLUME 4
ISSUE 2 MARCH – APRIL **ISSN: 2581-7876**

Abstract: Dexamethasone is a long-actingsynthetic adrenocortical steroid with low molecular weight.It readily crosses maternal placenta barrier and accumulates in the fetal tissues then impeding deleterious impact to the developing fetal pancreas.The aim of this study is to determine the histomorphological teratogenic changes on pancreasof the developing fetal pancreas following prenatal exposure to varied doses of dexamethasone in albino rats.40 albino rats weigh up between 150 to 300 grams were used as the albino rats experimental model.12 dams for the LDGgetting 0.5 mg/kg/bwt/day, 12 MDGgetting 2 mg/kg/bwt/day, and 12 HDGreceiving 4 mg/kg/bwt/day. Each group had a sum of 12 rats in first trimester (TM1), second trimester (TM2) and third trimester (TM3).Morphologically, there was decrease in the size and the number of islets clusters per field, decreased vascularization and increased stromal tissues deposition especially in high dexamethasone groups (4gms/Kg/Bwt). The number of acini per cluster, were significantly decreased ($P<0.05$), in both high and medium dexamethasone dose when treated in the first and second trimesters (TM1 and TM2) respectively These teratogenic outcomes of dexamethasone on the embryological fetal pancreas were also noted to be time and dose dependent with the most adverse effects werein the first and second trimester (TM1 and TM2) respectively in all the dexamethasone treated groups.This researchdisplays that high glucocorticoid levels during gestation have remarkable effects for the embryology of the fetal pancreas and especially in endocrine component, exocrine component and stromal tissue component of pancreas.

Keywords: dexamethasone, teratogenic, histomorphology, fetal pancreas

INTRODUCTION

Glucorticoids have numerous injurious effects to the developing organs of the developing fetuses including ;muscles, liver, kidneys, brain, lung, ,placenta, spleen and heart[1]. For instance, when dispensed during first trimester pregnancies leads to placenta insufficiency by inhibiting placental VEGF expression [1].In brain, leads to decline of the blood brain barrier permeability, reduction of fetal cerebral blood flow, hypoxia of brain, reduction in hippocampal sizeto learning and attention disorders[2,3]. In the kidneys, it causes shrinkingof nephron and glomerular of the kidney and lesseningof the glomerular number resulting glomerulosclerosisand hypertension[4,5,6]. Fetal treatmentby use of glucorticoids have in last four decade been in used for treatment of various disease during intrauterine period and newborns[1,7–9]. Dexamethasone have proved to be more effective than betamethasone in for preterm birth in enhancement of fetal lung surfactant production and maturation of the fetal lung, especially to mothers who are prone to preterm deliveries pregnancy(1). A onedose of 12 mg of dexamethasone is given to mothers at gestation period between 24 weeks and 34 weeks with ruptured membrane and 23 weeks to 34 weeks who are at risk of preterm deliveries and between one to 7 days before delivery of multiple pregnancies [10,11]. Clinically ,prenatal dexamethasone reduces neonatal death, acute respiratory diseases, severe neurological deficit and, necrotizing enterocolitis [4,8,10,12].

Dexamethasone its capabilityto cross the placenta have aidedin treatment of virilizing congenital adrenal hyperplasia (CAH) which is an autosomal recessive disorder of steroidogenesis, caused by lack of 21-hydroxylase[13,14]. The prenatal treatment of CAH is dispensed on 5th week of prenatal period, when genitalia are developing to stabilize androgen precursor[1,13,15]. Clinical studies on human have also shown that dexamethasone is clinically efficientin management of third-degree heart block[4,16],also a first line drug in management of Congenital cystic adenomatoid malformations [17,18]. Dexamethasone was also found to be effective in treatment ofperiventricular leukomalacia in low birthweight (≤1.75 kg) infants bydecreasing the risk [19]. Contrarywise, to its manyclinical importance's, dexamethasone have also numerous unwanted effects, this have been reported on researches done on animal and human studies to the fetus[20] .Dexamethasone use for instance contribute to multifarious metabolic effects like glucose intolerance, hyperglycemia which could have teratogenic effect to the pancreas of the developing embryo or fetus [21,22]. Studies done on animal and human have demonstratedthat prenatal dexamethasone

inhibitsthe metabolism of the developing fetus causinglow birth weight, intrauterine growth retardation, leanfetuses, raisedhypothalamopituitary adrenal axis activity, shrunkenbrain growth with prolongedmyelination and hypertension[2,3,23–26].

MATERIAL AND METHODS

Animals

Female nulliparous albino rats weighing between 150g to 300 grams were obtained from SAFARI animal biomedical department. They were housed in standard rat cages and subjected to 12-hour dark cycles under humid tropical conditions of 24°C. The rats were allowed unlimitedaccess to standard feed Rodent pellets obtained from UNGA Mills as institutedby American institute of nutrition (1977 (Unga feeds Kenya). and water ad libitum throughout the studytime. The rats were approvedwith the managingprinciples of laboratory animals' principles. Two females were hostedto one male albino rat and put into a cage overnight. The next day, the males were taken back to their specificcages.Vaginal smears were collected from the 40 mated females the next morning and pregnancy was determined by the presence of spermatozoa in the smears followed by vaginal wash 24 hours later to determine changes in estrous which will denote the first day of gestation (GD1)[27,28].These 40 dams were at randomassigned into two major groups of 36 dams as the experimental groupand 4 dams as the control group. 12 dams for the LDGreceived 0.5 mg/kg/bwt/day, 12 MDG received 2 mg/kg/bwt/day,12 HDG received 4 mg/kg/bwt/day and received each group 12 rats. These 12 dams in each group were further subdivided into three experimental group with 4 rats per group according to trimesters; first trimester (TM1), second trimester (TM2) and third trimester (TM3).

Feeding and Prenatal Dexamethasone dispensation

All experimental group received oral dexamethasone dissolved in normal saline via gastric gavage (Gauge 1.8 2R2 needle) and rodent pellets and water ad libitum between 8:00 am to 9: am [29].The control group received only the rodent pellets and water ad libitum between 8:00am to 9: am.The dexamethasone groups received(HDG 0.65mg/kg/d, MDG 7mg/kg/d, LDG 13 mg/kg/d) during the gestation period in first trimester, second trimester and third trimester. The dosage used in this study have been found to be comparable with human dose used during pregnancy (0.5-10mg/kg). The 12 albino rats in trimester 1 received dexamethasone treatment from day one of gestation all through to day 20; those in trimester two study category received dexamethasonetreatment starting day 7 all throughout to the last day of gestation day 20, while the 12 albino rats in trimester III start receiving the dexamethasone treatment from day 14 all through to- day 20 the last day of gestation.

In all cases, the pregnant rats were sacrificed on day 20th using carbon dioxide gas asphyxiation [30] using carbon dioxide asphyxia between 08:30 and 11:00 A.M.

Processing for light microscopy

After fixing in the 10% neutral buffered formalin for 24 hours the two fetal pancreases per sub group were dehydrated in an ascending concentration of alcohol (50%, 60%, 70%, 80%, 90%, 95% and 100% (absolute) each for one hour and cleared with cedar wood oil for 12 hours.

The sections were then infiltrated with paraplast wax for 12 hours and embedded in paraffin wax. Leitz sledge microtome was used to cut longitudinal and transverse thin sections 5-7µm thick from head to the tail regions of the fetal pancreas, floated in water at 370 then stuck onto glass slides using egg albumin, applied as thin film with a micro-dropper. 45 slides in each subgroup selected with systematic random sampling were then dried in an oven at 370 for 24 hours then stained with hematoxylin and eosin to demonstrate the general features of fetal pancreas components. Another 30 slides from each pancreas randomly selected were stained with Hematoxylin stainto demonstrate cellular components.

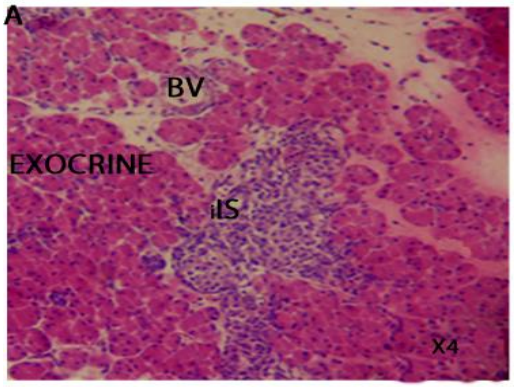
Ethical approval

All procedures were performed with approval of Albino rats Ethics Committee of Jomo Kenyatta University of Science and Technology. The albino rats were only used once in the experiment. They were all sacrificed using humane end points at the end of the study [29]

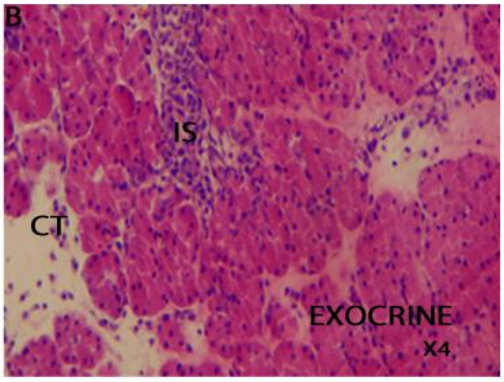
RESULTS

The Endocrine pancreas

When dexamethasone was administered in trimester one there was marked reduction in the number islet of Langerhans cluster (IS) per field (**figure 1)** - **photomicrographs B,C,D** for the LDG, MDG and HDG respectively as compared with the control **photomicrograph** A) this was also marked with significantreduction and disaggregation of the cellular densities of the various cellular components that make up the endocrine portion of the pancreas including the beta, alpha, delta and other endocrine cells in the dexamethasone treated groups LDG, MDG and HDG as compared with the control (C) (**figure 2) photomicrographs B,C,D** for the LDG, MDG and HDG respectively as compared with the control **photomicrograph** A.

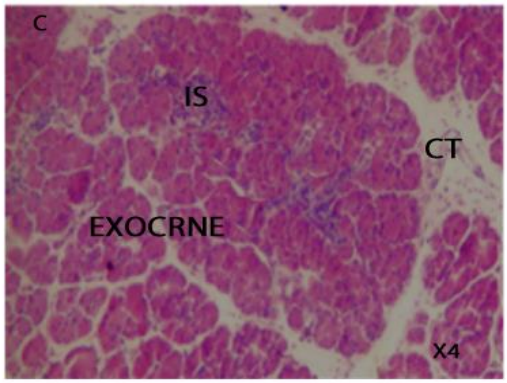


Photomicrograph A:pancreas of control rat stained with H&E showing:IS-well demarcated islets of langerhans;CT-connective tissue;BV-blood vessels

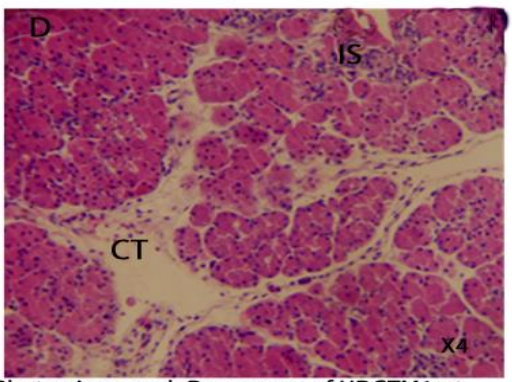


Photomicrograph B:pancreas of LDGTM1 rat stained with H&E showing:reduction in the in size of isletscluster (IS),CT-connective tissue;

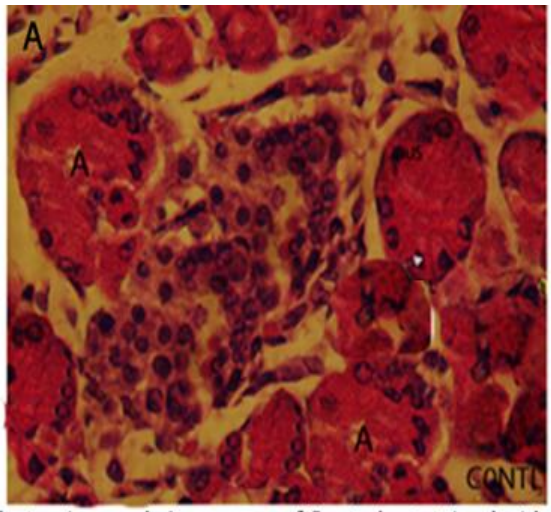
Fi
C



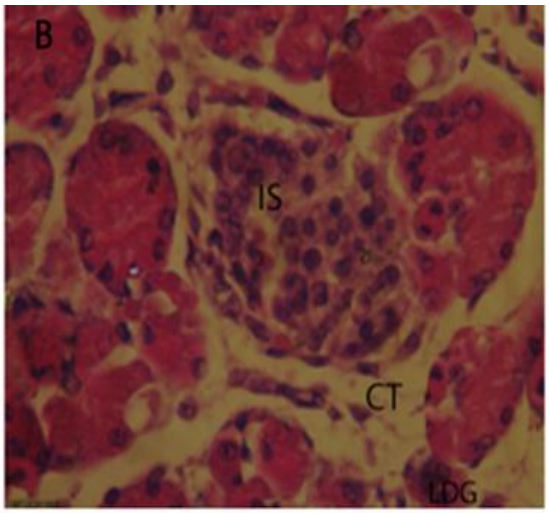
Photomicrograph C:pancreas of MDGTM1rat stained with H&E showing:more reduction in the size of islets cluster (IS), increased septations in CT-connective tissue;



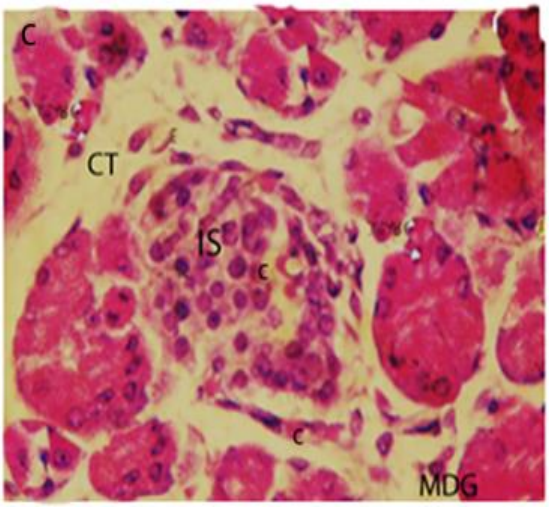
Photomicrograph D:pancreas of HDGTM1rat stained with H&E showing:Imore reduction in the size of islets cluster (IS), increased septations in CT-connective tissue;



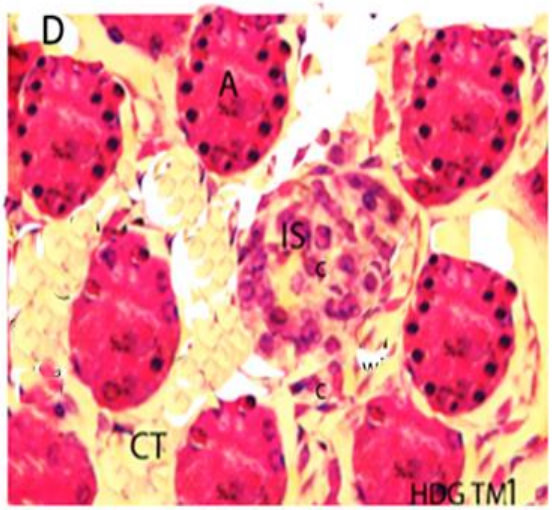
photomicograph A:pancreas of Control rat stained with H&E showing;CT-connective tissue ,*asterix-acinar cells,BV-blood vessels;IS-slets of Langrhan's;
A-acinus,c-capillaries,CA-centralacinar cell X40



photomicograph B:pancreas of LDGTM1 rat stained with H&E showing;CT-connective tissue ,acinar cells,
BV-blood vessels;IS-slets of
Langrhan's;A-acinus,c-capillaries,CA-cntrolacinar cell X40



photomicograph C:pancreas of MDGTM1 rat stained with H&E s
howing;CT-connective tissue ,acinar cells,BV-blood vessels;
IS-slets of Langrhan's;A-acinus,c-capillaries,CA-cntrolacinar cell X40

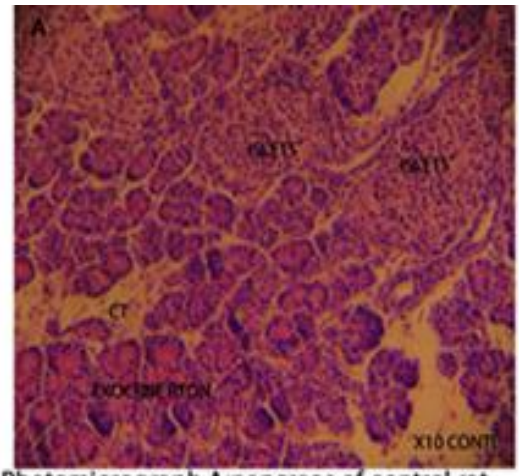


photomicograph D:pancreas of LDGTM1rat stained with H&E s
howing;CT-connective tissue ,acinar cells,BV-blood vessels;
IS-slets of Langrhan's;A-acinus,c-capillaries,
CA-cntrolacinar cell X40

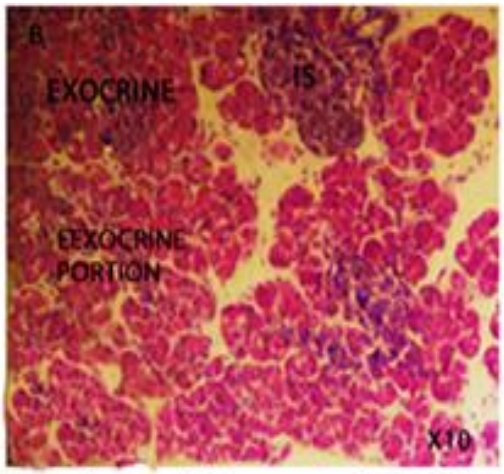
Figure 2: Photomicrograph of albino rat treated with dexamethasone A: Control; B: LDG, C: MDG &D: HDG at Trimester 1- (GD1-GD20)- (H & E X40)

The exocrine, the connective tissues and the duct system of the fetal pancreas when treated at TM_1

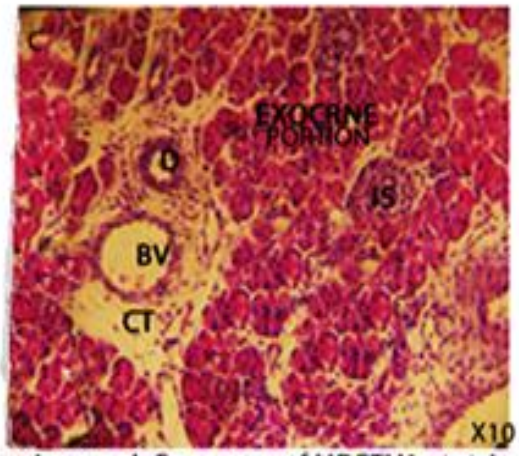
When dexamethasone was administered in trimester one there was decreased number of acini (A) (**figure 2)**-**photomicrographsB, C, D** for the LDG, MDG and HDG respectively as compared with the control photomicrograph **A**). There was also enlarged blood vessels, increased connective tissue septations (S) across the entire pancreas with increased number of ductal systems in the dexamethasone treated groups LDG, MDG and HDG as compared with the control (C). Photomicrographs B, C, and D for the LDG, MDG and HDG respectively as compared with the control photomicrograph A.



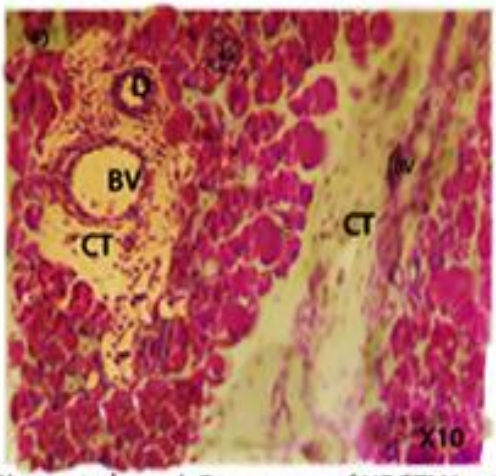
Photomicrograph A:pancreas of control rat stained with H&E showing: well demarcated islets langerhans;CT-connective tissue;



Photomicrograph B:pancreas of LDGTM1rat staine with H&E showing: reduction in the size of islets per pancreatic area (IS),CT-connective tissue;



Photomicrograph C:pancreas of MDGTM1rat stained with H&E showing: more reduction in the size of islets per pancreatic area (IS), increased septations in CT-connective tissue;BV-blood vessels,D- interlobular ducts



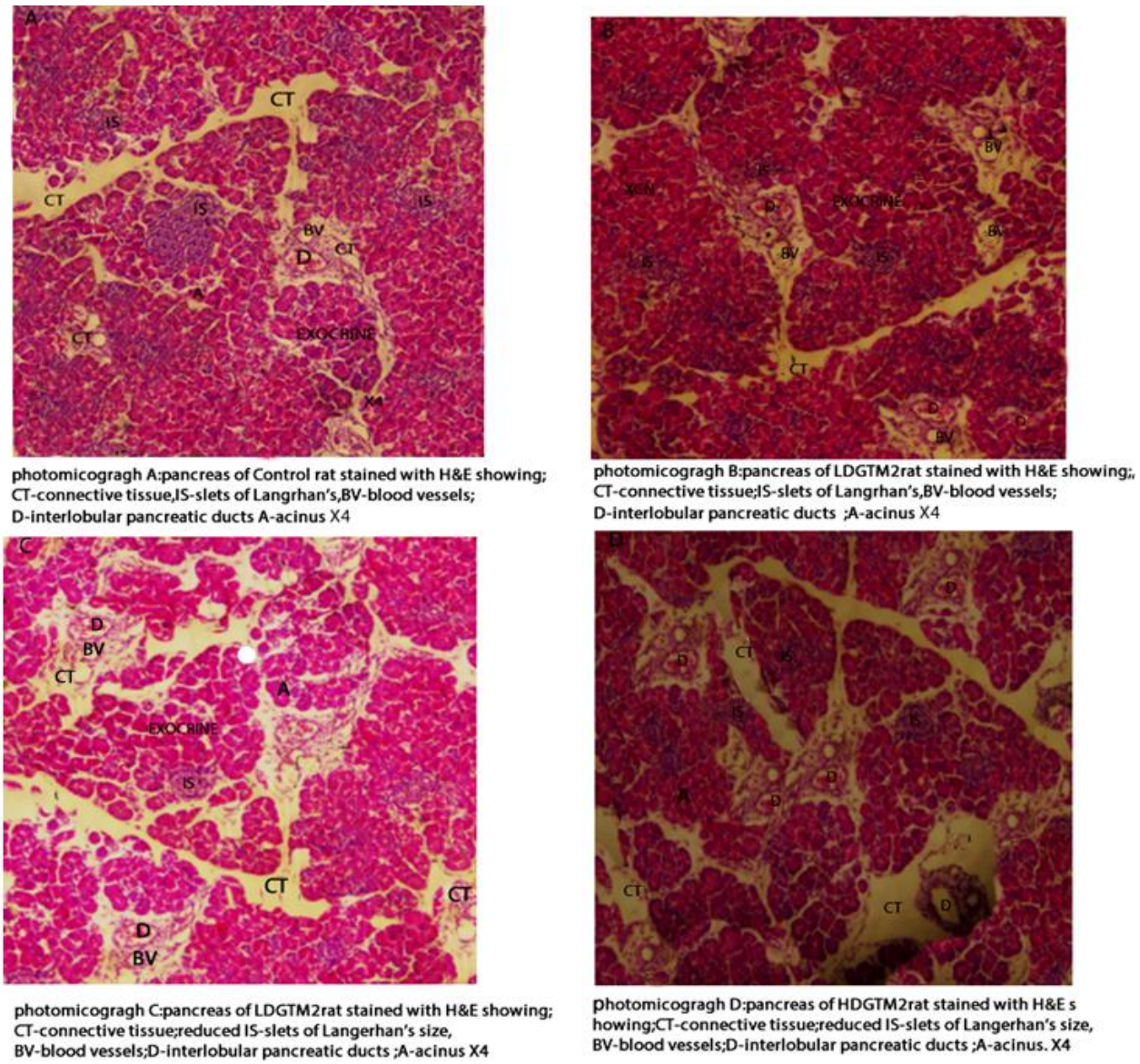
Photomapbgraph D:pancreas of HDGTM1 rat staine with H&E showing: further reduction in the size of islets per pancreatic area (IS), increased septations in CT-connective tissue

Figure3: Photomicrograph of albino rat treated with dexamethasone A: Control; B: LDG, C: MDG &D: HDG at Trimester 1- (GD1-GD20)- (H & E X40)

The trimester TWO (TM2) histomorphological findings on the fetal pancreas

The Endocrine pancreas

When dexamethasone was administered in trimester two, there was marked reduction in the number islet of Langerhans cluster (IS) per field (figure 4.5 - photomicrographs B, C, D for the LDG, MDG and HDG respectively as compared with the control photomicrograph A). This was also marked with significant reduction and disaggregation of the cellular densities of the various cellular components that make up the endocrine portion of the pancreas including the beta, alpha, delta and other endocrine cells in the dexamethasone treated groups LDG, MDG and HDG as compared with the control (C) as (figure 4.5),photomicrographs B,C,D for the LDG, MDG and HDG respectively as compared with the control photomicrograph A. B,C,D for the LDG, MDG and HDG respectively as compared with the control photomicrograph A.



photomicograph A:pancreas of Control rat stained with H&E showing; CT-connective tissue,IS-slets of Langrhan's,BV-blood vessels; D-interlobular pancreatic ducts A-acinus X4

photomicograph B:pancreas of LDGTM2rat stained with H&E showing;, CT-connective tissue;IS-slets of Langrhan's,BV-blood vessels; D-interlobular pancreatic ducts ;A-acinus X4

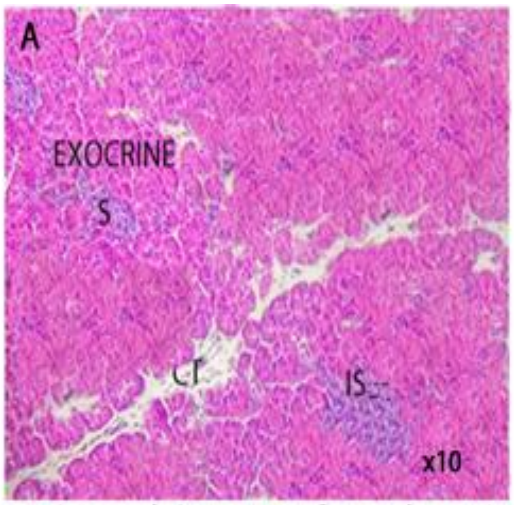
photomicograph C:pancreas of LDGTM2rat stained with H&E showing; CT-connective tissue;reduced IS-slets of Langerhan's size, BV-blood vessels;D-interlobular pancreatic ducts ;A-acinus X4

Photomicograph D:pancreas of HDGTM2rat stained with H&E s howing;CT-connective tissue;reduced IS-slets of Langerhan's size, BV-blood vessels;D-interlobular pancreatic ducts ;A-acinus. X4

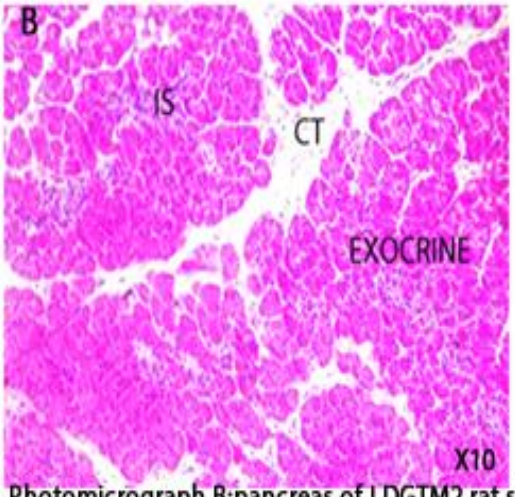
Figure 1*:* Photomicrograph of albino rat treated with dexamethasone A: Control; B: LDG, C: MDG &D: HDG at Trimester 2- (GD7-GD20)- (H&EX4).

The exocrine, the connective tissues and the duct system of the fetal pancreas when treated at TM_2

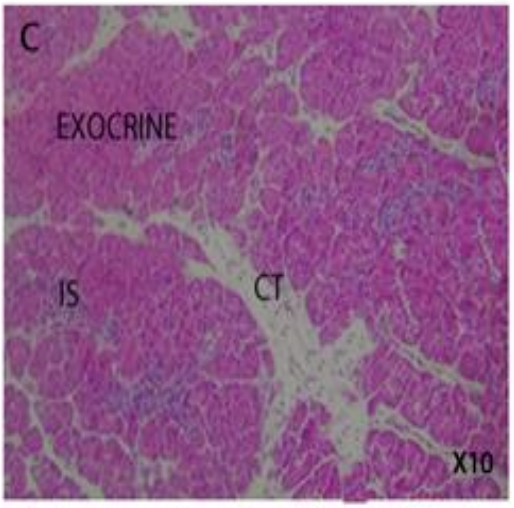
When dexamethasone was administered in trimester one increase in septations of connectivetissue (CT)) across the entire pancreas and reduction in number of acini (A) (**figure 4 - photomicrographs B, C, D** for the LDG, MDG and HDG respectively as compared with the control photomicrograph **A**). There was also enlarged blood vessels with increased number of ductal systems in the dexamethasone treated groups LDG, MDG and HDG as compared with the control (C) (**figure 4),** photomicrographs B,C,D for the LDG, MDG and HDG respectively as compared with the control photomicrograph A



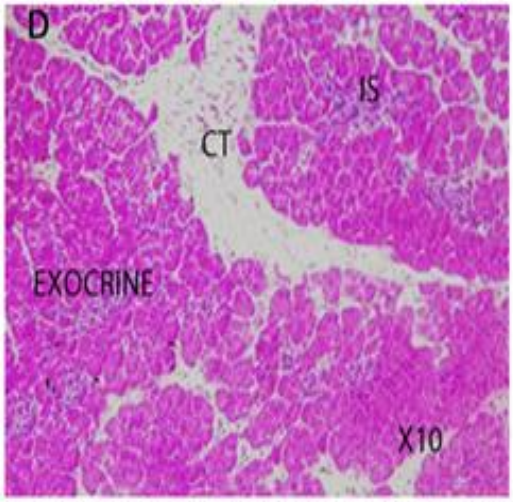
Photomicrograph A:pancreas of control rat stained with H&E howing:IS-well demarcated islets of langerhans;CT-connective tissue;



Photomicrograph B:pancreas of LDGTM2 rat stained with H&E showing:reduction in the size of islets per pancreatic area (IS),CT-connective tissue;



Photomicrograph C:pancreas of MDGTM2 rat stained with H&E showing:more reduction in the size of islets per pancreatic area (IS), increased septations in CT-connective tissue;

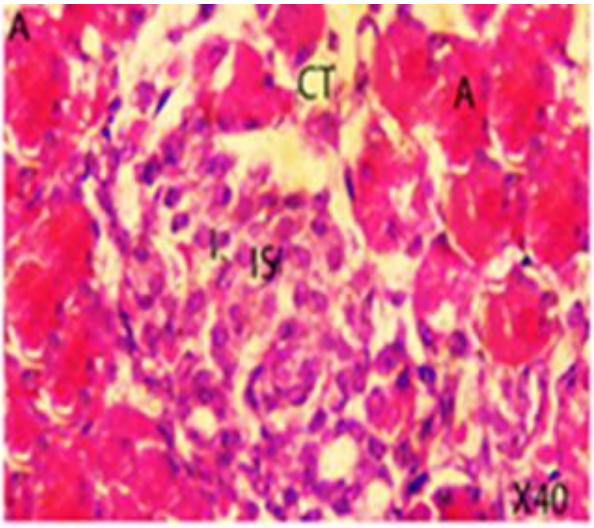


Photomicrograph D:pancreas of HDGTM2 rat stained with H&E showing:further reduction in the size of islets per pancreatic area (IS), increased septations in CT-connective tissue;

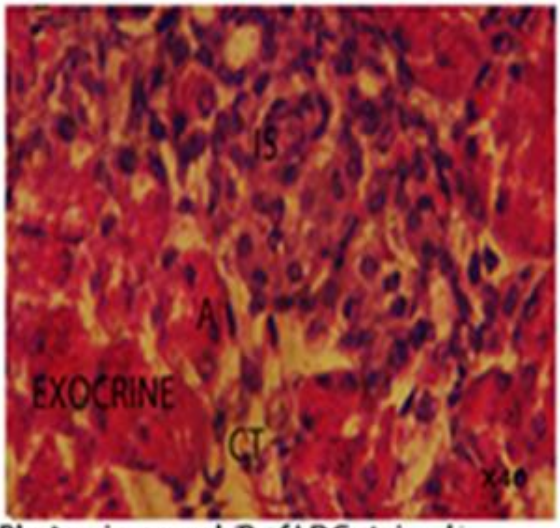
Figure5: photomicrograph of albino rattreated withdexamethasone A: Control; B: LDG, C: MDG &D: HDG at Trimester 2- (GD7-GD20)- (H & E X10)

The endocrine, the exocrine, the stromal tissues and the duct system of the fetal pancreas when treated at TM_2 X40

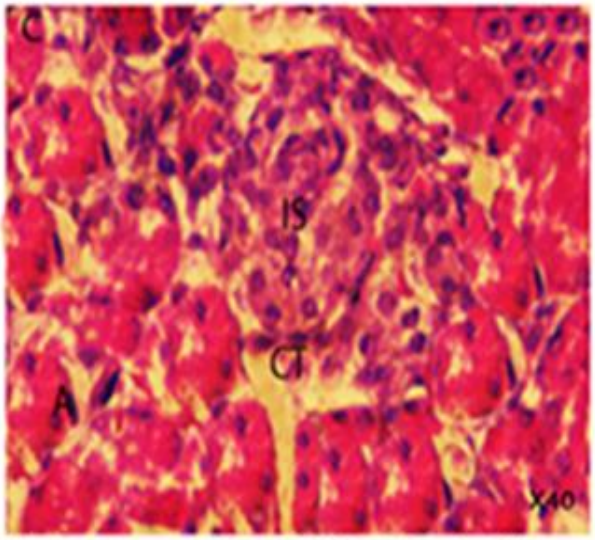
When dexamethasone was administered in trimester one there was decreased number of acini (A) (**figure 6 - photomicrographs B, C, D** for the LDG, MDG and HDG respectively as compared with the control photomicrograph **A**). There was also enlarged blood vessels, increased connective tissue septations (S) across the entire pancreas with increased number of ductal systems in the dexamethasone treated groups LDG, MDG and HDG as compared with the control (C) (**figure 6**), photomicrographs B, C, D for the LDG, MDG and HDG respectively as compared with the control photomicrograph A.



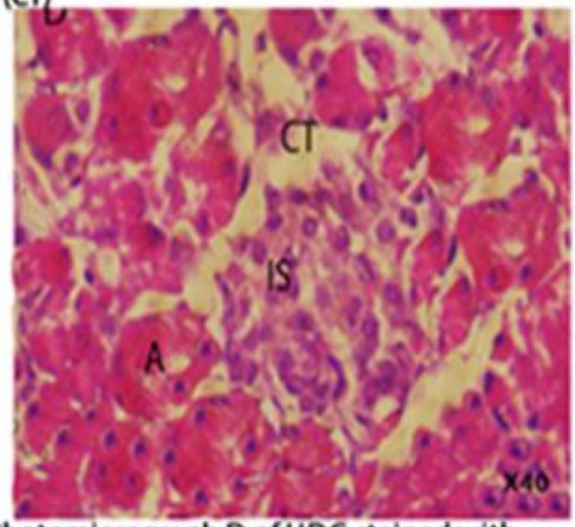
Photomicrograph A of control stained withH&E showing acidophilic acini(A),well defined islets of Langerhans (IS) and many lobules of different sizes and shapes bound in connective tissue stroma (CT)



Photomicrograph B of LDG stained with H&E showing reduced number of acini (A),reduced number of islets of Langerhans (IS) and many lobules of different sizes and shapesa bound in connective tissue stroma (CT)



Photomicrograph C of MDG stained with H&E showing further reduced number of acini (A),more reduced number of islets of Langerhans (IS) and increased lobules of different sizes and shapes bound in connective tissue stroma (CT)



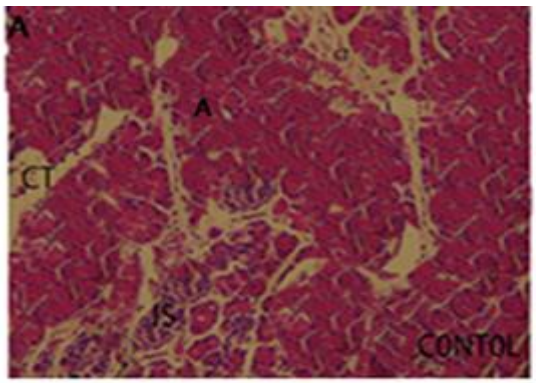
Photomicrograph D of HDG stained with H&E showing most reduced number of acini (A),most number of islets of Langerhans (IS) and marked increased lobules of different sizes and shapes bound in connective tissue stroma (CT)

Figure7: Photomicrograph of albino rat treated with dexamethasone A: Control; B: LDG, C: MDG &D: HDG at Trimester 2- (GD7-GD20)- (H & E X10) at Trimester 2- (GD7-GD20)- (H & E X40)

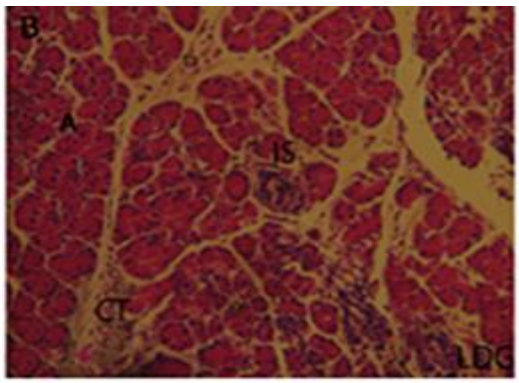
The trimester three (TM_3) histomorphological findings on the fetal endocrine pancreas

The Endocrine pancreas

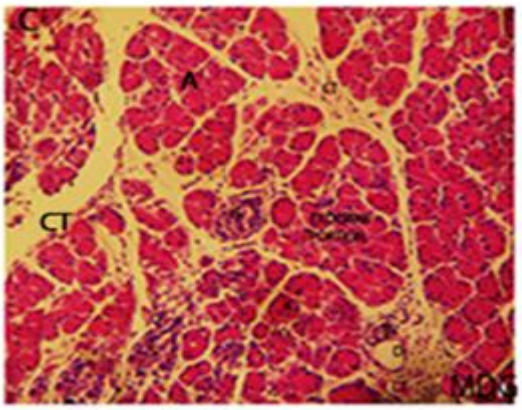
When dexamethasone was administered in trimester three there was marked reduction in the number islet of Langerhans cluster (IS) per field [**figure 4.8) - photomicrographs B,C,D** for the LDG, MDG and HDG respectively as compared with the control photomicrograph A) this was also marked with significant reduction and disaggregation of the cellular densities of the various cellular components that make up the endocrine portion of the pancreas including the beta, alpha, delta and other endocrine cells in the dexamethasone treated groups LDG, MDG and HDG as compared with the control (C) as can be seen in the figure 4.8, photomicrographs B,C,D for the LDG, MDG and HDG respectively as compared with the control photomicrograph A.



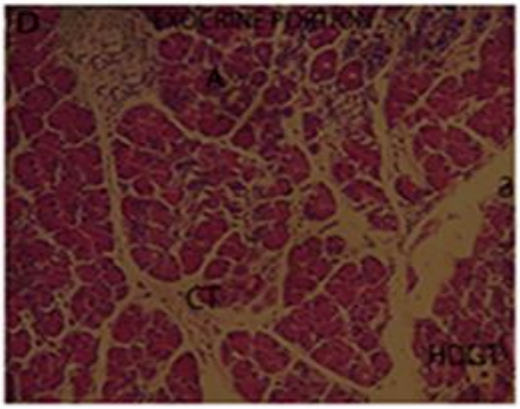
Photomicrograph A of control stained withH&E showing acidophilic acini (A),well defined islets of Langerhans (IS) and many lobules of different sizes and shapes bound in connective tissue stroma (CT)



Photomicrograph B of LDG stained with H&E showing reduced number of acini(A),reduced number of islets of Langerhans (IS) and many lobules of different sizes and shapesa bound in connective tissue stroma (CT)



Photomicrograph C of MDG stained with H&E showing further reduced number of acini (A),more reduced number of islets of Langerhans (IS) and increased lobules of different sizes and shapes bound in connective tissue stroma (CT)



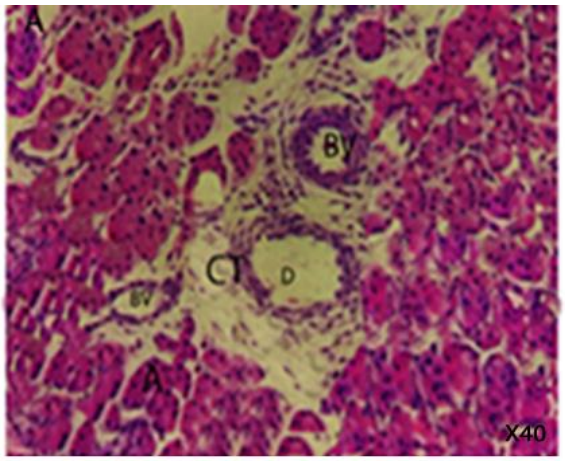
Photomicrograph D of HDG stained with H&E showing most reduced number of acini(A),most number of islets of Langerhans (IS) and marked increased lobules of different sizes and shapes bound in connective tissue stroma (CT)

Figure 8: Photomicrograph of albino rat treated with dexamethasone A: Control; B: LDG, C: MDG &D: HDG at Trimester 3 (GD_{14}-GD_{20})- (H & E X 10)

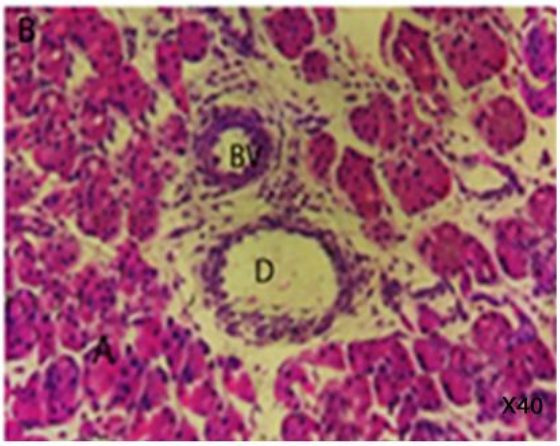
The exocrine, the connective tissues and the duct system of the fetal pancreas when treated at TM_3

When dexamethasone was administered in trimester three there was marked reduction in number of acini clusters (A), increase of the connective tissue deposition (CT) is obvious in the surroundings of the ducts (D) and the

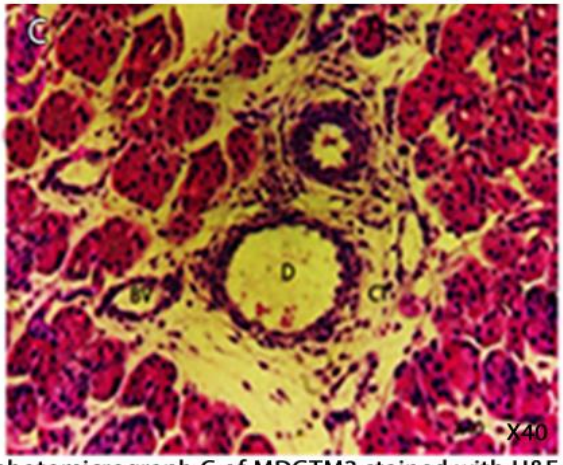
thicken vascular wall of blood vessels (BV) in the dexamethasone treated groups LDG, MDG and HDG as compared with the control (C) (**figure9)**.



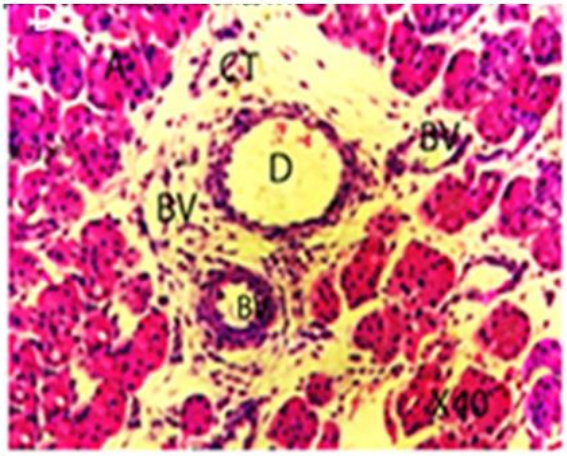
Photomicrograph A of control stained with H&E showing; acini(A)that are well stained ; connectve tissue stroma (CT)consting of thin wall ducts(D);blood vessels(BV)



PhotomicrographB of LDGTM3 stained with H&E showing; acini(A)that are well stained ;increases depositions in connectve tissue stroma (CT)consting of thickened wall of ducts(D);blood vessels(BV)



photomicrograph C of MDGTM3 stained with H&E showing; reduced number of acini(A)that are well stained ;increased depositions and septations in connectve tissue stroma (CT)consting of tthickened wall of ducts(D);blood vessels(BV)



photomicrograph D of HDGTM3 stained with H&E showing; more reduced number of acini (A) ;increased depositions and septations in connectve tissue stroma (CT)consting of tthickened wall of ducts (D);blood vessels(BV)

Figure 9: Photomicrograph of albino rat treated with dexamethasone A: Control; B: LDG, C: MDG &D: HDG**at Trimester 3- (GD14-GD20)- (H & E X40).**

DISCUSSION

The current findings on the teratogenic effects of dexamethasone on the histomorphological components of both the parenchymal and stromal tissues of the fetal pancreas have showed marked reduction in the number of islets of Langerhans cluster per field (**figure 4.2- figure 4.9**). This was also marked with significant reduction and disaggregation of the cellular densities of the various cellular components that make up the endocrine portion of the pancreas including the beta, alpha, delta and other endocrine cells in the dexamethasone treated groups LDG, MDG and HDG as compared with the control. Also, there was also enlarged blood vessels, increased connective tissue septations (S) across the entire pancreas with increased number of ductal systems in the dexamethasone treated groups LDG, MDG and HDG. This is agreement with a study that reported that glucorticoids increases angiogenesis by reducing endothelial cell migration hence impairing extracellular matrix and reduce proliferation in

various cell types [30]. This mechanism can be explained by the fact that dexamethasone particularly hinders migration of vascular smooth muscle in rats as opposed in human [31,32,33]

The results of this study also showed that cells in the pancreas were composed of smaller acinar cells with scanty but larger and deeply stained nuclei. The numerical numbers of the acinar clusters per field with their corresponding numerical cell count per acini were found to be statistically reduced across all dexamethasone treated groups ($P<0.05$). The nuclei of both acini and ductal cells varied in sizes and shapes with many showing mitotic figures. (**Fig 4:2-Fig 4:9**) [34,35]. This fact disagrees with Morisset, *et al.* who reported that glucocorticoid administration was associated with hyperplasia and hypertrophy of the pancreas in suckling rats but suppressed deoxyribonucleic acid (DNA) synthesis in recently weaned rats while maintaining the hypertrophic effects [36].

Conclusion

In conclusion the study has established that prenatal exposure to dexamethasone is teratogenic to the developing fetal pancreas and these teratogenic outcomes are dose and time dependent. The critical dose of dexamethasone teratogenicity was found to be the high and medium dexamethasone dose when exposed at first and second window period. Such effects of dexamethasone on pancreas in children born to mothers may predispose to pancreatic disorders in postnatal period. LDG and MDG trimester three had no significant outcomes except when administered on high doses.

The most vulnerable window period for dexamethasone teratogenicity was however established to be the first trimester while the most critical dose was **4mg/kg/bwt.**

Conflicts of interest

The author declares that they have no conflict of interest.

Acknowledgement

The authors express gratitude, to the tremendous support from the department of Human Anatomy, College of Health Science (COHES) JomoKenyatta University of Agriculture and Technology.

REFERENCESS,

1. Singh RR, Cuffe JSM, Moritz KM. Short and long term effects of exposure to natural and synthetic glucocorticoids during development. 2012;57–69.
2. Ilg L, Klados M, Alexander N, Kirschbaum C, Li S. Long-term impacts of prenatal synthetic glucocorticoids exposure on functional brain correlates of cognitive monitoring in adolescence. Sci Rep [Internet]. 2018;(December 2017):1–11.Available from: http://dx.doi.org/10.1038/s41598-018-26067-3
3. Noorlander CW, Tijsseling D, Hessel EVS, Vries WB De, Derks JB, Visser GHA, et al. Antenatal Glucocorticoid Treatment Affects Hippocampal Development in Mice. 2014;9(1):1–7.
4. Hui L, Bianchi DW. Prenatal pharmacotherapy for fetal anomalies : a 2011 update. 2015;31(7):735–43.
5. Neuhaus W, Schlundt M, Fehrholz M, Ehrke A. Multiple Antenatal Dexamethasone Treatment Alters Brain Vessel Differentiation in Newborn Mouse Pups. 2015;1–21.
6. Ortiz LA, Quan A, Zarzar F, Weinberg A, Baum M. Prenatal Dexamethasone Programs Hypertension and Renal Injury in the Rat. 2003;328–34.
7. Shang H, Meng W, Sloboda DM, Li S, Ehrlich L, Plagemann A, et al. Effects of Maternal Dexamethasone Treatment Early in Pregnancy on Glucocorticoid Receptors in the Ovine Placenta. 2015;22(5):534–44.
8. Abrantes MA, Valencia AM, Bany-mohammed F, Aranda J V, Kay D. Combined antenatal and postnatal steroid effects on fetal and postnatal growth , and neurological outcomes in neonatal rats. 2019;11(3):1697–710.
9. Braun T, Meng W, Shang H, Li S, Sloboda DM, Ehrlich L, et al. Early Dexamethasone Treatment Induces Placental Apoptosis in Sheep. 2015;22(1):47–59.
10. Scientist MS, Registrar JBD, Gerard H, Visser A. Antenatal corticosteroid therapy and fetal behaviour :

a randomised study of the effects of betamethasone and dexamethasone. 1997;104(November):1239–47.
11. Attawattanakul N. Effects of Antenatal Dexamethasone on Respiratory Distress in Late Preterm Infant : A Randomized Controlled Trial. 2015;23(1):25–33.
12. Sheen, J. M., Hsieh, C. S., Tain, Y. L., Li, S. W., Yu, H. R., Chen, C. C., Huang, L. T. (2016). Programming Effects of Prenatal Glucocorticoid Exposure with a Postnatal High-Fat Diet in Diabetes Mellitus. International journal of molecular sciences, 17(4), 533. doi:10.3390/ijms17040533.13. Griffiths SK, Hons B, Bs BM, Campbell JP, Hons M, Frca M. Placental structure , function and drug transfer. 2015;15(2):84–9.
14. Rennick GJ. Use of systemic glucocorticosteroids in pregnancy : Be alert but not alarmed. 2006;(August 2005):34–6.
15. Rivkees SA. Dexamethasone Therapy of Congenital Adrenal Hyperplasia and the Myth of the " Growth Toxic " Glucocorticoid. 2010;2010(Figure 1).
16. Breur JMPJ, Visser GHA, Kruize AA, Stoutenbeek P, Meijboom EJ. Treatment of fetal heart block with maternal steroid therapy : case report and review of the literature. 2004;(June):467–72.
17. Rhen, T., & Cidlowski, J. A. (2005). Anti-inflammatory action of glucocorticoids--new mechanisms for old drugs. The New England journal of medicine, 353(16), 1711–1723. https://doi.org/10.1056/NEJMra050541
18. Fan D, Wu S, Wang R, Huang Y, Fu Y, Ai W, et al. Successfully treated congenital cystic adenomatoid malformation by open fetal surgery. 2017;0(December 2016):0–4.
19. Kemp MW, Newnham JP, Challis JG, Jobe AH, Stock SJ. The clinical use of corticosteroids in pregnancy. Hum Reprod Update [Internet]. 2016;22(2):240–59. Available from: https://academic.oup.com/humupd/article-abstract/22/2/240/2457843
20. Chen YC, Huang YH, Sheen JM, Tain YL, Yu HR, Chen CC, et al. Prenatal Dexamethasone Exposure Programs the Development of the Pancreas and the Secretion of Insulin in Rats. Pediatr Neonatol [Internet]. 2017;58(2):135–44. Available from: http://dx.doi.org/10.1016/j.pedneo.2016.02.008
21. Rennick, G. J. (2006) 'Use of systemic glucocorticosteroids in pregnancy: (August 2005), pp. 34–36. doi: 10.1111/j.1440-0960.2006. 00219.
22. Nyirenda MJ, Welberg LA, Seckl JR. (2001) Programming hyperglycaemia in the rat through prenatal exposure to glucocorticoids-fetal effect or maternal influence? J Endocrinol. 2001; 170:653–660.
23. Whitelaw A, Thoresen M. Antenatal steroids and the developing brain. Arch Dis Child Fetal Neonatal Ed [Internet]. 2000 Sep;83(2):F154--7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10952714
24. Moraes E de F, Wanderley Teixeira V, Teixeira ÁAC, da Silva WE, Batista APC, de Lemos AJJM. Effect of the Treatment with Dexamethasone, for 10 and 15 Days, on the Fertility in Induced Rats to Polycystic Ovaries, by Constant Illumination. Int J Morphol. 2008;26(3):659–63.
25. Ogueh, J. Jones, H. Mitchell, J. Alaghband-Zadeh, M.R. Johnson, (1999) Effect of antenatal dexamethasone therapy on maternal plasma human chorionic gonadotrophin, oestradiol and progesterone, Human Reproduction, Volume 14, Issue 2, February 1999, Pages 303–30614(2) 303–306. doi: 10.1093/humrep/14.2.303
26. Chakraborty S, Islam S, Saha S, Ain R. Dexamethasone-induced Intra- Uterine Growth Restriction impacts NOSTRIN and its downstream effector genes in the rat mesometrial uterus. Sci Rep [Internet]. 2018;(May):1–13. Available from: http://dx.doi.org/10.1038/s41598-018-26590-3
27. Shedrack I, Nwocha C, Ikechukwu J, Diseases R. A NEW AND SIMPLE METHOD OF CONFIRMATORY DETECTION OF MATING IN ALBINO RATS [Rattus norvegicus). 2006;3:527–30.
28. Hamid HY, Abu Z, Zakaria B. Reproductive characteristics of the female laboratory rat. 2013;12(19):2510–4.
29. Son, D. J., Lee, G. R., Oh, S., Lee, S. E., & Choi, W. S. (2015). Gastroprotective efficacy and safety evaluation of scoparone derivatives on experimentally induced gastric lesions in rodents. Nutrients, 7(3), 1945–1964. https://doi.org/10.3390/nu7031945
30. Varish Ahmad, Prospective of extracellular matrix and drug correlations in disease managementAsian Journal of Pharmaceutical Sciences,https://doi.org/10.1016/j.ajps.2020.06.007
31 Pross, C., Farooq, M. M., Lane, J. S., Angle, N., Tomono, C. K., Xavier, A. E., Freischlag, J. A., Collins, A. E., Law, R. E., & Gelabert, H. A. (2002). Rat and human aortic smooth muscle cells display differing migration and matrix metalloproteinase activities in response to dexamethasone. Journal of vascular surgery, 35(6), 1253–1259. https://doi.org/10.1067/mva.2002.123332

32. Grgić G, Fatusić Z, Bogdanović G. [Stimulation of fetal lung maturation with dexamethasone in unexpected premature labor]. Med Arh [Internet]. 2003 [cited 2019 Jun 15];57(5–6):291–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15022581
33. Yahi D, Ojo NA, Mshelia GD. Influence of Dexamethasone on Some Reproductive Hormones and Uterine Progesterone Receptor Localization in Pregnant Yankasa Sheep in Semiarid Zones of Nigeria. 2017;2017(Cl).
34. Rafacho A, Cestari TM, Taboga SR, Boschero AC, Bosqueiro JR. High doses of dexamethasone induce increased beta-cell proliferation in pancreatic rat islets. Am J Physiol EndocrinologyMetab2009;296(4):E681-9.http://www.ncbi.nlm.nih.gov/pubmed/19158320
35. Elsnosy E, Shaaban OM, Abbas AM, Gaber HH, Darwish A. Effects of antenatal dexamethasone administration on fetal and uteroplacental Doppler waveforms in women at risk for spontaneous preterm birth. Middle East Fertil Soc J [Internet]. 2017;22(1):13–7. Available from: http://dx.doi.org/10.1016/j.mefs.2016.09.007
36. Morisset, J., & Jolicoeur, L. (1980). Effect of hydrocortisone on pancreatic growth in rats. The American journal of physiology, 239(2), G95–G98. https://doi.org/10.1152/ajpgi.1980.239.2.G95
38. Dumortier O, Theys N, Ahn M-T, Remacle C, Reusens B. Impairment of Rat Fetal Beta-Cell Development by Maternal Exposure to Dexamethasone during Different Time-Windows. Keating D, editor. PLoS One [Internet]. 2011 Oct 3 [cited 2019 Jun 4];6(10):e25576. Available from: https://dx.plos.org/10.1371/journal.pone.0025576