Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Karbala / College of Veterinary Medicine

Physiology, Biochemistry and pharmacology Department

Physiological and Histopathological Study of Glutathione Effect Before and During Early Pregnancy Rats Exposed to D-galactose

A thesis

Submitted to the Council of The College of Veterinary Medicine, University Of Karbala In Partial Fulfillment of The Requirements For the Degree of Master of Science In Veterinary Medicine/ Physiology

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2021 A.D **1443 A.H**

بسم الله الرحمن الرحيم

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Dedication

I dedicate this work

To whom ...Who I carry his name proudly... and his affection the reason for my success.....My Dear father Ghassan Al-rubai

To my angel in life... To the meaning of love and compassion, who rejoice the heart for her existence .....My darling mother Rukea Al-fatlawi

To emeritus and my strength, who removed the thorns from my path to pave my way to the science and gave me love, cooperation and support.....My Dear Brother Mustafa ..... My Dear sisters Huda , Zainab and Zahra

Zinah 2021

Acknowledgments

First of all I'd like to praise and offer my gratitude to my creator, who gave me health and strength to complete this work.

I would like to thank the Department of physiology , College of veterinary Medicine/University of Karbala for providing all facilities and guidance.

My deepest gratitude and great respect, to my supervisor ***Prof. Wefak Mohammed ALbazi*** *and* ***Dr. Alaa Hussein ALsafy*** *for whom insufficient words could meet my gratitude, his patience, guidance and constant encouragement I received from him throughout the study and during writing this thesis.*

I also would like to thank all the members of my family and friend , who encouraged me and stood by my side to complete my studies.

Zinah 2021

Summery

This study is carried out to investigate the protective role of Glutathione on the reproductive system induced by D-galactose overload in female rats. injected intraperitoneally (IP) with 100 mg/kg B .W of D-galactose and GSH for 45 day.

Twenty female (20) rats were divided into four groups (20/5), the first group was injected with normal saline for 45 day and served as control group(G1), rats in the second group were injected intraperitoneally (IP) with 100 mg/kg B .W of D-galactose (G2). While animals of the third group were (G3) injected intraperitoneally (IP) with 100 mg/kg B .W of Glutathione , rat in the fourth group (G4) were injected intraperitoneally (IP) with 100 mg/kg B .W of D-galactose and Glutathione .

Fasting blood samples were collected by cardiac punctures technique at 45 day of experimental for measuring: biomarker of oxidant and antioxidant including serum Glutathione (GSH) , Malondialdehyde (MDA) concentrations ,Superoxide dismutase (SOD) and Catalase (CAT) .serum hormones follicle stimulating hormone (FSH) ,Luteinizing hormone (LH),Estrogen hormone and Progesterone hormone serum Lipid profile ,Low density lipoprotein (LDL),High density lipoprotein (HDL),Triglycerides(TAG),Total Cholesterol(TC) and Very Low density lipoprotein (VLDL),D-serum Glucose

The results before pregnant revealed to a significant ($p \leq 0.05$) increase in Glucose level in G2 compared with G1,G3 and G4 also showed a significant increase in MDA,TC,TAC,LDL-C,VLDL-C in G2 group comparing with other groups ,serum estrogen showed a significant ($p \leq 0.05$) increase in G2 comparing with control group. And showed significant decrease in HDL-C ,SOD.CAT and GSH in G2 comparing with G1,G3 and G4 groups , serum FSH showed a significant decrease

in G2 group comparing with G1 and G3groups ,while serum LH in G3 and G4 comparing with other groups, also serum progesterone calculated showed a significant decrease in G2 group when comparing with G1, G3 and G4groups .

While results in our study after seven day of pregnant showed a significant decrease ($p \leq 0.05$) in serum LH and FSH level in G2 group comparing with G1.G3 and G4 groups ,also a significant decrease in serum estrogen and progesteron in G2 group comparing with other groups .The research revealed to a significant decrease in the level of HDL-C. GSH,SOD and CAT concentration in the G2 group comparing with other groups, while showed a significant($p \leq 0.05$) increase in the serum level of progesterone, TC, TAG, LDL-C,VLDL-C and MDA concentration in G2 group compering with G1,G3 and G4 groups .

The histological change, it was the effect of D-galactose on the ovary, it affected the rate of ovulation and the number of mature follicles in the ovary. On the other hand, it had no effect on the implantation of embryos in the uterus of pregnant rats, and we noticed in the histological sections of the ovaries and uterus of the groups that were treated with glutathione, the results were in contrast to D-galactose only, the ovary was at the peak of its activity, and the uterus and implantation were in the correct form.

In conclusion, the results of this study confirm the protective role of GSH against the D-galactose over load in the reproductive system and hormones before and during early pregnancy

List of Abbreviations

Abbreviation	Meaning
µl	microliter
AGE	Advanced glycation endproduct
B.W	body wight
C	Centigrade
CAT	Catalase
CHOL	Cholesterol
CL	Corpus luteum
Cm	centimeter
D-gal	D-Galactose
DNA	Deoxyribonucleic Acid
DPX	Dextrin plasticizer xylene
EDTA	Ethylen ediaminetetra acetic acid
E-H.S	Eosin-Hematoxilin Stain
ELISA	Enzyme-Linked Immunosorbent Assay
FSH	Follicle stimulating hormone
Gal-1-p	Galactose-1-phosphate
GALT	Gut Associated lymphoid tissue
GGT	γ-Glutamyltransferase
Gm	Gram
GR	glutathione reductase
GSH	glutathione
H&E	Hematoxylin & Eosin

Hb	Hemoglobin
HDL	High -density lipoprotein
I.P	Intraperitoneal injection
kg	Kilogram
L.S.D.	Least significant difference
LDL	low-density lipoprotein
LH	Luteinizing hormone
MDA	Malondialdehyde
mg	milligram
mg/dl	milligrams per decilitre
mg/kg	Milligram per kilogram
mIU/ml	Microliter international unit per milliliter
NADPH	Nicotinamide adenine dinucleotide phosphate
ng/ml	Nanograms per millilitre
nm	Nanometer
PAS	Periodic Acid Schiff's stain
pg/ml	Picograms per millilitre
pH	potential of hydrogen
r / min	Revolutions per minute
ROS	reactive oxygen spices
sec	Seconds
SOD	superoxide dismutase
TAG	Triglyceride
TBA	Thiobarbituric acid
TC	Total Cholesterol
UDP	Uridine diphosphate galactose

VLDL	very-low-density lipoprotein
W/V	Weight /volume

List of contents

List of tables

List of figures

1.Introduction

D-galactose is a monosaccharide sugar that could be metabolized if administrated within a normal range of concentrations. also, the most critically important pathway is the Leloir pathway, the main pathway of galactose metabolism **(Qi & Tester 2019)** , D-galactokinase and glucose-1-phosphate uridyl-transferase, a substrate in glycolysis, rapidly metabolize to galactose via four consecutive enzymes. Altered D-galactose formed with glucose-1-phosphate should facilitate glucose conversion into proteins and fat **(Zhou *et al*., 2015).** Although D-gal. could be converted to glucose in normal concentration **(Chen *et al*.,2010;Eszenyi *et al.*, 2011)** , while in high levels of D-galactose contributed to metabolic pathway disruption. It is oxidized by galactose oxidase enzyme into aldehydes and hydrogen peroxide, is also converted to galactitol that accumulates in the cell and lead to osmotic stress and toxicity to reactive oxygen spices (ROS) in the tissue **(Kumar *et al.*, 2011; Sharma *et al.*, 2014; Makarov *et al.*, 2014;Zhou *et al.*, 2015; Bo-Htay *et al.*,2018).**D-galactose generates ROS in vivo during its metabolism by interacting readily with amino group present in nucleic acid, protein and lipids in order to form advanced- glycation end products , Advanced Glycation End products (AGE) lead to ROS generation **(Munch *et al*.,2012;Zhang *et al.*, 2013).**

D-galactose has been used for inducing aging and express associated changes in physiology of body, like decreased antioxidant enzyme activity, increased oxidative stress, cognitive dysfunction, mitochondrial dysfunction, accumulation of metabolites and diminished immune response **(Aydin *et al*.,2016; Jeremy *et al*., 2017)** , numerous studies have been conducted to assess the aging mechanisms of the brain **(Sadigh-Eteghad *et al.*, 2017;Shwe *et al*., 2018)** and heart **(BoHHtay *et al*., 2018)**, based on a D-gal aging of animal model, also could be used for modeling pancreatic, kidney **(El-Far *et al.*, 2020)** and liver **(Saleh *et al.*, 2019).** In addition, there have been a number of studies implicating D-gal-induced skin aging **(Sukoyan *et al*., 2018; Sulistyoningrum *et al.*, 2019; Umbayev *et al*.,2020).**

Some studies have used D-galactose to induce Premature Ovarian Insufficiency (POI) in laboratory animals **(Dovom *et al*.,2020).** A systematic review in 2019 showed that using different doses of galactose during the prenatal or postnatal period produced different ovarian follicle deficiency outcomes in rodents **(Sozen *et***

***al*.,2019;El Bakly *et al*.,2020).** Exposure to D-galactose during the prenatal period decreases the count of small ovarian follicles (primordial and primary) **(Hagen-Lillevik *et al*.,2021),**

Glutathione (L-y-glutamyl-L-cysteinyl-glycine) is a tripeptide widely distributed in animal and plant tissues that fills an important role in regulating the ability of cells to withstand exposure to oxidizing metabolites, drugs, and xenobiotics **(Brechbuhl, 2008).** Together with its related enzymes, comprises a system that maintains the intracellularreducing environment and acts as primary defense against excessive generation of harmful ROS**(Nimse & Pal 2015).** The oxygen radical scavenging activity of glutathione directly facilitates ROS neutralization and the repair of ROS-induced damage**(Adeoye *et al*.,2018).** In addition to its antioxidant activity, glutathione has many physiological functions including detoxification of xenobiotics, modulation of redox-regulated signal transduction, regulation of cell proliferation, and immune responses **(Espinosa-Diez *et al*.,2015).**

Antioxidant is generated in all tissues, but in the liver in disproportionately significant amounts. Despite the fact that GSH is synthesized from three constitutive amino acids (cysteine, glutamate, and glycine), cysteine is the restricting factor**(Bhattacharyya *et al*.,2014).** The biological oxidative effects of free radicals on macromolecules are controlled by a spectrum of enzymatic and nonenzymatic antioxidants **(Sharma *et al*.,2019).**

Glutathione exists in the thiol-reduced (GSH)2 and disulfide oxidized (GSSG) forms **(Zhou *et al* .,2017).** Eukaryotic cells have three major reservoirs of GSH **(Monroy *et al* .,2016).** Almost 90% of cellular GSH are in the cytosol, 10% in the mitochondria, and a small percentage in the endoplasmic reticulum **(Raturi *et al* .,2007).**

Glutathione (GSH) is an important intracellular peptide with multiple functions ranging from antioxidant defense to modulation of cell proliferation. GSH is synthesized in the cytosol of all mammalian cells in a tightly regulated manner**(Lushchak ,2012).** The major determinants of GSH synthesis are the availability of cysteine, the sulfur amino acid precursor, and the activity of the rate-limiting enzyme, g-glutamylcysteine synthetase In the liver, major factors that determine the availability of cysteine in diet, membrane transport activities of the three sulfur amino acids cysteine, cystine and methionine, and the conversion of methionine to cysteine via the trans-sulfuration pathway **(Forman *et al*.,2018)**

Glutathione is produced in the cytosol and transported into organelles (nucleus,

endoplasmic reticulum and mitochondria) via specialized transporters. Two novel GSH transporters recently characterized for the mitochondria were functionally linked to regulation of mitochondrial respiration, thereby providing evidence for the critical role of GSH in maintaining mitochondrial function. **(Sreekumar *et al* .,2021).**

The present study was examined to estimation the possible protective role administration of GSH against adverse effects of D-galactose has been known to be one of the generate oxidative stress in the uterus and ovary before and during early stage of pregnant in the rats ,by the following

1-Estimation some hormones (LH ,FSH, estrogen and progesteron)

2- Evaluate the oxidative parameters (MDA ,SOD ,CAT and GSH)

3- Estimation of serum Lipid profile (TC,TAG,HDL-C,LDL-C and VLDL-C)

4- Estimation level of Glucose

5-Examin histopathological of the uterus and ovary.

2. Review of Literature

2.1.1 D-Galactose

Chemical formula :- $C_6H_{12}O_6$

D-galactose is an aldohexose , a reducing sugar that occurs naturally in the body and in many foods such as milk, butter, cheese, yogurt, honey, beets, plums, cherries, figs, and celery **(Azman & Zakaria2019).**

Galactose was first identified in milk by Louis Pasteur in 1856, who denominated it as ‘lactose’. Only later, it was named ‘galactose’ from the Greek word ‘galakt’, which means ‘milk’. Galactose is a natural aldohexose that, like most sugars, occurs more frequently in nature in its D-configuration.

For a healthy adult, the maximal recommended daily dose of galactose is 50 g and most of it can be metabolized and excreted from the body within about 8 h after ingestion **(Morava ,2014).** However, at high levels, it can be converted into aldose and hydroperoxide under the catalysis of galactose oxidase, resulting in the generation of reactive oxygen species (ROS) **(Wu *et al*. 2008).** D-galactose is a reducing sugar, and when it accumulates in the body, it can react with the free amines of amino acids in proteins and peptides to form a Schiff base, an unstable compound **(Farajdokht *et al* .,2021).**

The biological importance of galactose, however, goes beyond its importance as a nutrient and a metabolite **(Bo-Htay *et al*.,2018).**Galactose appears to have been selected by evolutionary pressure to also exert a crucial structural role. Indeed, despite the fact that it differs from glucose in the configuration of the hydroxyl group at the carbon-4 position, **(Coelho *et al*.,2015).** galactose has a myriad of specific functional and structural roles in living organisms that cannot be exerted by glucose. **(Szilagyi,2019).**

D-galactose is ubiquitous in bacteria, plants, and animals **(Lee et al.,2008).** D-galactose was first used in the study of aging by researchers in China where they discovered that injections of D-galactose may reduce longevity in rodents **(Ho *et al*.,2003).**

2.1.2 Galactose absorption and metabolism

studies have shown that galactose and glucose share a common intestinal transport carrier. This transport carrier has a greater affinity for glucose than galactose **(Wright *et al*.,2003),** which may explain why galactose absorption is inhibited by glucose but glucose absorption is unaffected by galactose**(de Jongh *et al*.,2008)** When galactose is absorbed along with glucose, serum galactose concentrations are considerably lower than when the same amount of galactose is consumed without glucose**(Liu *et al*.,2005).** The absorption of galactose may also be reduced by leptin and b3-adrenergic receptor agonists **(Liu *et al*.,2015).**

The major pathway for galactose metabolism is considered to be the conversion of galactose to glucose by Gal-1-P, UDP-gal, and glucose-1-P. This conversion of galactose to glucose has been observed to be enhanced by glucose both via an increase in GALT activity in the liver and also via insulin action **(Zeng *et al*.,2010).** Aldose reductase also enhances the breakdown of galactose by catalyzing the NADPH-mediated conversion of galactose to galactitol**(Liu *et al*.,2015).** This pathway, however, is likely to be important in galactose toxicity via accumulation of galactitol **(Leslie,2003).**

2.1.3 Oxidative stress and D-galactose:

The term oxidative-stress is that characterized imbalances by the development of reactive oxygen species (ROS) related free radicals and the antioxidant protection system. Hydroxyl radical ($OH^{\bullet -}$), superoxide radical ($O_2^{\cdot -}$), and nitric oxide (NO) are the most common reactive oxygen species. **(Wu *et al*., 2014).**

Oxidative stress is a common pathophysiological phenomenon that causes damage to cells by continuous releasing of ROS that largely originated from biotic and abiotic stressors exposure **(Apel and Hirt, 2004).** The common features of various reactive oxygen species are cellular damage building blocks or biomolecules such as lipids, DNA, proteins **(Ray *et al*., 2012;Halliwell and Gutteridge, 2015).** Oxidative stress is one of the main factors responsible for organ damage through oxidative injury due to lipid peroxidation marker production, which has caused several diseased conditions including diabetes, cardiovascular dysfunction, iron overload, iron deficiency anemia, cancer, reproductive dysfunction, Al-Zheimers disease and aging **(El-shishtawy *et al.*, 2016; Wongiaikam *et al.*, 2016; Imam *et al*., 2017).**

Experimentally induced oxidative stress include exposure to 0.5 - 1% H_2O_2**(AL-doseri and Khudair, 2016),** methionine overload **(Al-bazii and Khudiar,2017),** iron overload **(Khudair and naji, 2012),** high fructose diet **(AL-agele and Khudair,2016)**, acrylamide **(Khudiar and Hussein,2017),** and high cholesterol diet **(Khudair,2000).** Recently, D-galactose **(Bai *et al*.,2017),** D-galactoseamine **(Li *et al*., 2018),** monosodium glutamate **(Rosa *et al*., 2018)** and lipopolysaccharid **(Eren *et al*., 2018)** are regarded as oxidative stress promoters.

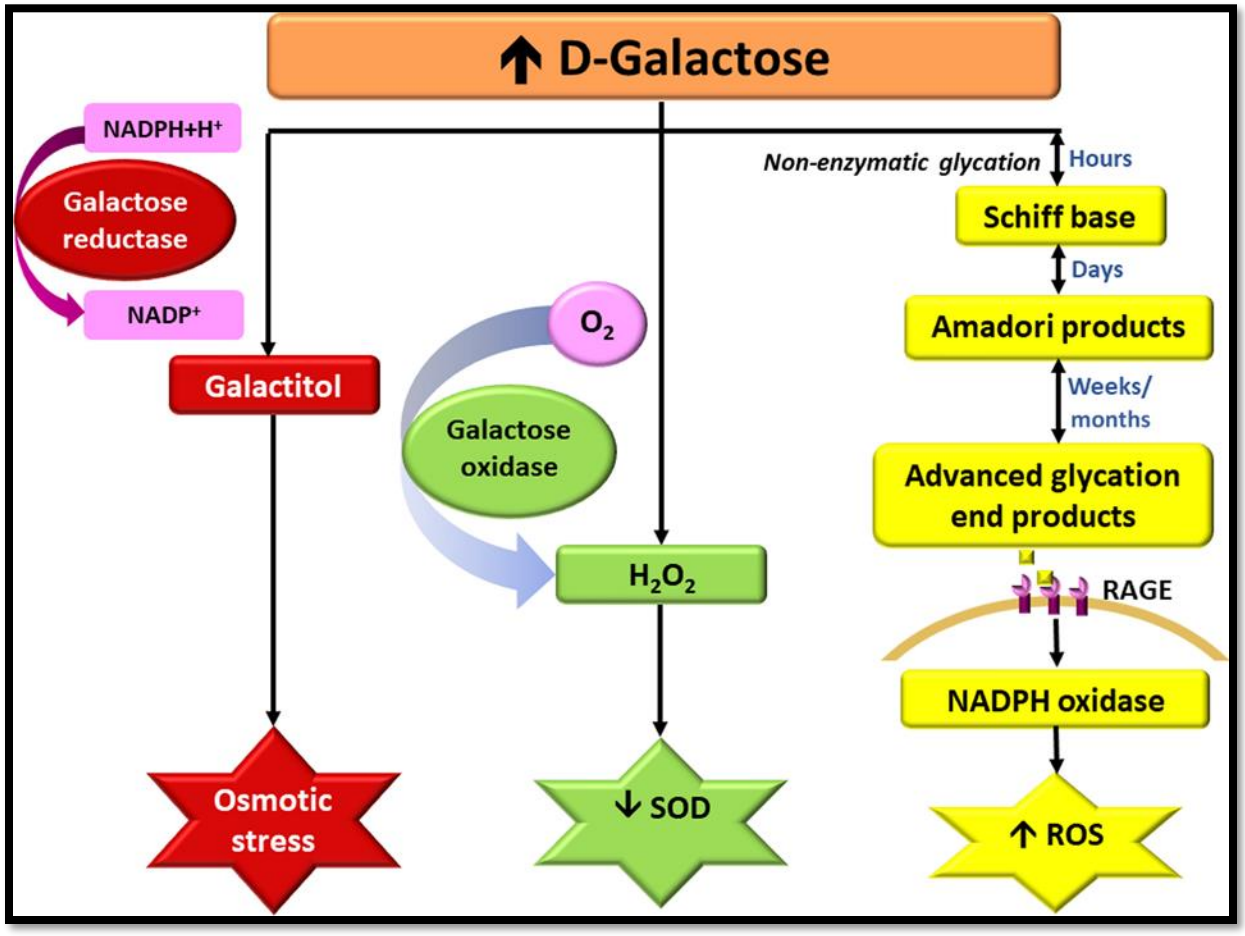


Figure 2.1: Induces oxidative stress by D-galactose. (Bo-Htay *et al*.,2018).

Excess d -galactose is reduced by galactose reductase to form galactitol which can lead to osmotic stress. Additionally, high level of d -galactose can be oxidized by galactose oxidase to yield hydrogen peroxide; increased hydrogen peroxide causes a decrease in antioxidant enzymes (SOD). Furthermore, d -

galactose can initiate non-enzymatic glycation reactions to form advanced glycation end products (AGE s) after weeks or months. When AGE s reacts with their receptors (RAGE), ROS production occurs through NADPH oxidase activation. H_2O_2, hydrogen peroxide; SOD superoxide dismutase; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species.

2.1.4 Aging:

D-galactose causes aging changes related to natural aging processes, such as shorter lifespan, neurodegeneration and cognitive dysfunction, advanced end-product glycation (AGE) formation and oxidative stress, and transcriptional gene changes **(Cardoso *et al*., 2015; Xia *et al*., 2015).**

Several studies explain that aging symptom mediated by D-gal could lead to oxidative stress (OS) triggered by elevation in D-gal metabolism **(Hao, *et al.*, 2014;Zhou *et al.*, 2015).** D-galactose-induced models of aged rats have been commonly used in the study of aging **(Zhang *et al.*, 2009).** D-gal administration for long periods of time will result in an enzymatic overload that impairs the natural ability of the body to catalyze galactose into glucose, resulting in an increase in galactitol and activation of aldose reductase. This causes cell oxidation damage **(Ullah *et al.*, 2015;Budni *et al.*, 2016)** and form AGE **(Munch *et al.*, 2012).** The production and accumulation of AGEs has been found to increase of ROS synthesis by acting as the interacting AGE (RAGE) receptor and increase process of aging **(Munch *et al.*, 2012;Zhang *et al.*, 2013; Wang *et al.*, 2014).** ROS caused oxidative stress is considered the main factor contributing to aberrates signaling pathway that contribute eventually to the process of aging **(Phull *et al.*, 2018).**

2.1.5 D-Galactose influence on the female reproductive system:-

In mammals, female reproductive capacity is negatively related to age, and the female reproductive system is faster to show overt physiological signs of aging than other body systems **(Velarde & Menon2016).** It has been found that giving rats D-galactose (gal) causes signs of aging **(Chen *et al.*, 2018).** Since then, D-gal injection has been used extensively to create anti-aging research,**(Li *et al.*, 2005).**Complex biological processes involving molecular, cellular, and organic changes are called aging, as a result of age-related physiological disturbances is a great deal of disorder such as imbalance and pathology.**(Sitzmann *et al.*, 2008)** Administration of D-Galactose may disrupt the estrous cycle and cause tissue damage to the uterus and ovaries**(Ahangarpour *et al.*, 2016).**

Young women with galactosemia experience ovarian failure at a very early age raising concern about the ovarian toxicity of galactose. While galactose may be present in the diet as a monosaccharide, it is predominantly derived from cleavage of the disaccharide lactose within the intestin **(Liu *et al.*, 2005).**

The toxic effect of galactose or its metabolites within the ovary became apparent from the observations of extremely early ovarian failure (before age 30 years) in women with galactosemia (genetic absence of the enzyme galactose-1-phosphate uridyl transferase) **(Nyhan *et al.*,2020).**

2.2.1 Pregnancy

Pregnancy is a period of intense physical and physiological changes, which is accompanied by certain changes in the maternal organism from fertilization to childbirth and during which the embryo and then the fetus develops in the maternal uterus **(Samir *et al.*,2018).** It is characterized by physiological changes related to the development and growth of the fetus, maternal adaptation to the pregnancy, preparation of the mother at childbirth, maintenance of maternal homeostasis and preparation for breastfeeding**(Kovacs,2015).**

Indeed pregnancy exposes too many complications that can be related to an alteration of oxidative stress that is also associated with the appearance of several pathologies during pregnancy**(Osol & Moore 2014).**

The process of embryogenesis is the basis for creating the final shape of an adult

(Levin,2012), and this process does not form Simply from the growth of a preformed miniature embryo in size, it is an enormous dynamic process characterized by a great deal of movement Cell movement morphogenetic and tissue reorganization, the fetus begins as a single cell is the fertilized zygote, which undergoes many repetitive divisions (mitochondrial mitosis)**(Almansa *et al*.,2020).** These cells are known as blastomers because of their rapid development (which leads to a progressive reduction in size) and the way they are generally organized to produce the final shape for the fetus . after which a mass called the morula is formed consisting of Cell 16 . **(Coward and Wells, 2013).**

The mass of the inner cells is called the inner cell mass, from which the fetal tissues and a group of peripheral cells arise A single layer of rapidly dividing cells surrounds the first, called the mass cell outer Trophoblast **(Cochard , 2012).**

2.2.2 Implantation:-

implantation is a dynamic developmental event that involves a series of physical and physiological interactions among the blastocyst trophectoderm and various endometrial cell-types, including the luminal and glandular epithelial and stromal cells **(Aplin and Kimber, 2004;Lim and Dey, 2009).** Implantation proceeds through three stages: apposition, adhesion/attachment, and penetration**(Cha *et al*.,2012).** During the apposition stage, the trophectoderm comes into close proximity with the luminal epithelium **(Aplin & Ruane 2017).** With uterine lumen closure, firm attachment between the trophectoderm and luminal epithelium is initiated. In mice, the attachment reaction occurs on day 4 of pregnancy at midnight, coinciding with a localized increase of endometrial vascular permeability**(Ye,2020).** After the attachment reaction, the implanting embryo initiates penetration through the luminal epithelium into the stromal bed**(Cha *et al*.,2013),**Therefore, implantation involves multiple cell–cell interactions between the blastocyst and the uterus under the primary influence of ovarian steroids**(Paria *et al*.,2002).**

Successful implantation requires synchronization between the acquisition of implantation competency by the blastocyst and a receptive state in the uterine endometrium**(Wang et al.,2005;Wang and Dey, 2006).**

These two events are precisely regulated by maternal hormones, in particular, ovarian estrogen and progesterone **(Arck et al.,2007).** Molecular and genetic evidence indicates that ovarian hormones together with locally produced signaling molecules, including cytokines, growth factors, homeobox transcription factors, lipid mediators and morphogen genes, function through autocrine, paracrine and juxtacrine interactions to specify the complex process of implantation **(Dey *et al*., 2004).**

The crosstalk between the blastocyst and the uterus can only occur during a brief period, namely the ‘‘window of implantation’’ **(Achache & Revel 2006).**

In response to the implanting embryo, the surrounding uterine stroma undergoes cellular transformation, a process known as decidualization , to accommodate embryonic growth and invasion**(Owusu-Akyaw et al.,2019).**

2.3.1 Glutathione

The tripeptide glutathione (GSH) (L-y-glutamyl-L-cysteinyl-glycine) is made up of cysteine, glutamic acid, and glycine. A c-glutamyl linkage and a sulphydryl (–SH) group are two structural characteristics of GSH **(Calabrese *et al*.,2017).** GSH is well-known for its many physiological activities, including xenobiotic compound detoxification and the antioxidation of reactive oxygen species (ROS) and free radicals. (**Lushchak,2014).** The chemical formula for is Glutathione **$C_{10}H_{17}N_3O_6S$** .

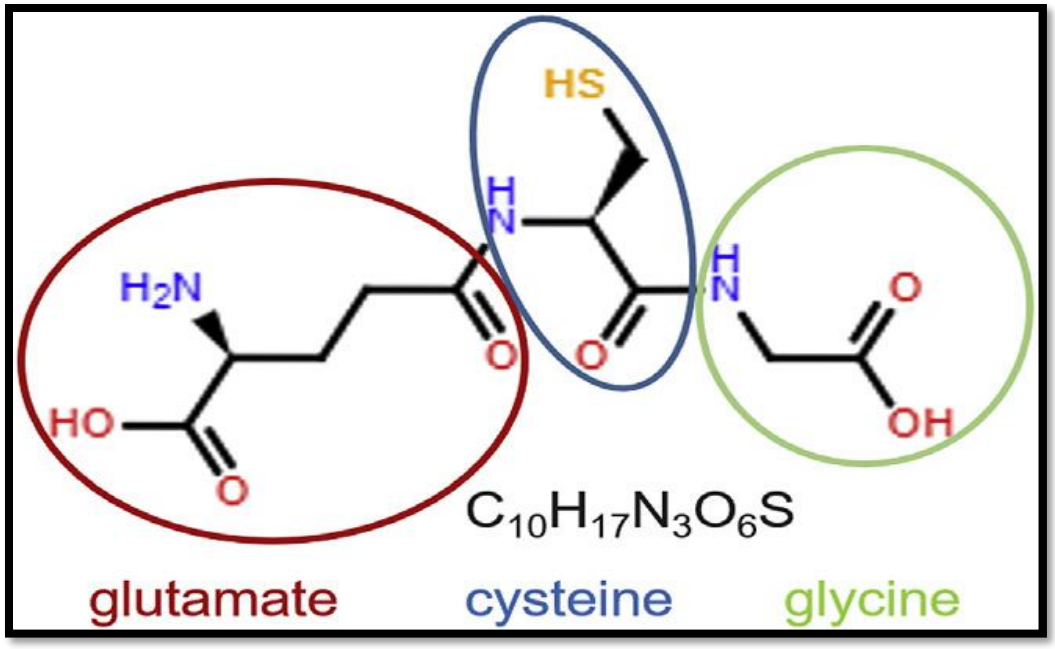


Fig. 2.2 Glutathione structure. Glutathione (GSH) is a tripeptide consisting of three amino acids: glutamate (red), cysteine (blue) and glycine (green) and is present in most mammalian tissues and cells. GSH serves as a major antioxidant, reactive oxygen species (ROS) scavenger, detoxification agent and signaling molecule. (Wang & Asmis 2020).

Glutathione (GSH) is the most abundant intracellular thiol, protects cellular components from oxidation and is maintained in a reduced state by glutathione reductase (GR).**(Jobbagy *et al*., 2018).**

Glutathione is a tripeptide widely distributed in animal and plant tissues that fills an important role in regulating the ability of cells to withstand exposure to oxidizing metabolites, drugs, and xenobiotics **(Al-Gubory *et al*.,2010).** Its biochemistry and biological role in the maintenance of thiol groups, redox status of proteins, removal of hydro peroxides, transport of amino acids, and detoxication of foreign compounds have been extensively reviewed in several books and articles**(Boušová & Skálová, 2012) .**

Glutathione is used in a widely rang in pharmaceutical compound and the GSH has used in food additives**(Gülçin *et al*.,2016).** well Glutathione have a low

molecular weight thiol, is one of the most important antioxidants and metal chelators **(Aslani & Ghobadi2016).** tripeptide glutathione (GSH, -L-glutamyl-L-cysteinylglycine) is essential for cellular defense against reactive oxygen species.

2.3.2 Glutathione biosynthesis

Glutathione is synthesized intracellularly by the consecutive actions of g-glutamylcysteine (reaction 1) and GSH (reaction 2) synthetase:

L-Glu+L-Cys+ATPUL-g-Glu−L-Cys+ADP+Pi (1)

L-g-Glu-Cys+Gly+ATPUGSH+ADP+Pi (2)

Cysteine is usually the limiting substrate in the synthesis of GSH. Intracellular GSH is exported from most cells, but it is not significantly taken up by cells under normal conditions **(Danilov, 2008)** . Once outside of the cell, the g-glutamyl bond of GSH may be cleaved by the membrane bound g-glutamyl transpeptidase whose active site is on the outside of some cells/organs. Transpeptidase is found in the kidney, choroid plexus, lymphocytes, biliary duct, ciliary body, intestine and pancreas. The product of the reaction is a g-glutamyl enzyme which can accept an amino acid to form g-glutamyl amino acid. **(Pastore *et al*.,2003).** After transport, the g-glutamyl amino acid is cleaved by g-glutamyl cyclotransferase to yield free amino acid and 5-oxoproline (a cyclic form of glutamate). 5-Oxoproline is ring opened by 5-oxoprolinase to give glutamate. The biosynthetic enzymes, together with these latter three enzymes, form the g-glutamyl cycle **(Njålsson,2005)** . One of the best acceptor amino acids for transpeptidase is cystine; thus, its product is g-glutamylcystine **(Franco *et al*.,2007).** This may be transported into certain cells, such as kidney, and reduced to cysteine and g-glutamylcysteine **(Lu, 2009).** Cysteine can be used to synthesize GSH using reactions (1) and (2) or be used for other cellular needs. g-glutamylcysteine can be used directly by GSH synthetase (reaction 2) to form GSH, bypassing the first enzyme. These series of reactions constitutes the alternative or salvage pathway of GSH biosynthesis **(Zhang et al.,2005).**

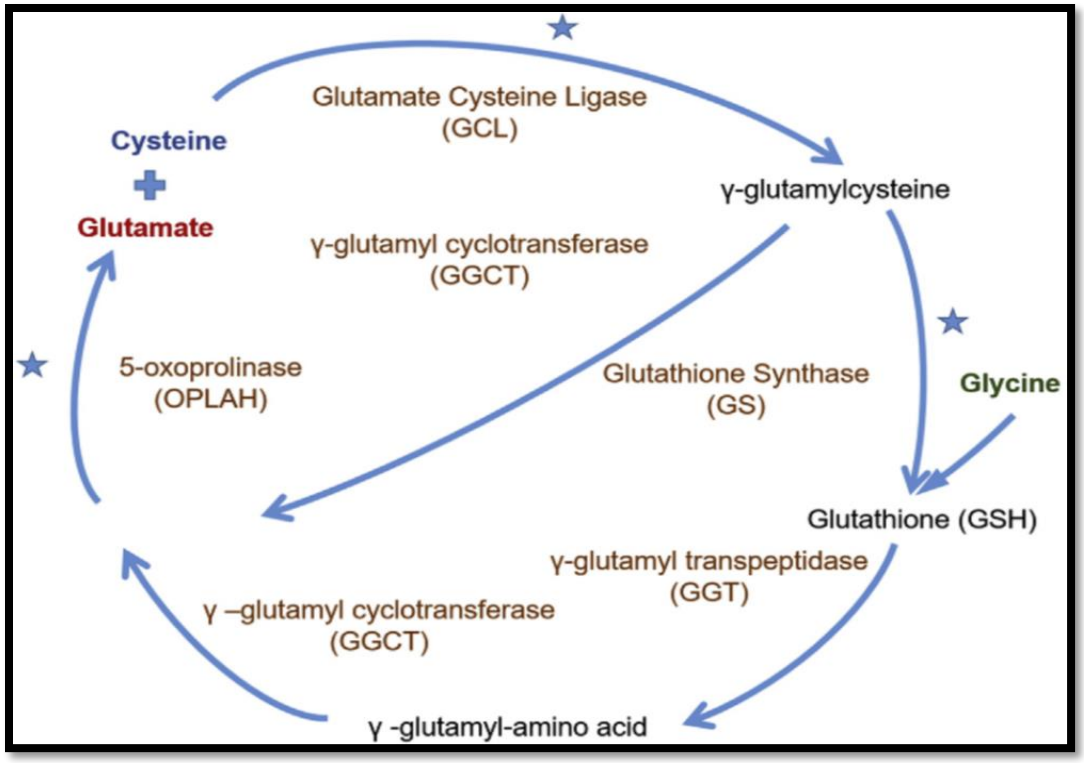


Fig. 2.3 Glutathione Synthesis and Metabolism. The rate-determining step in GSH synthesis is the formation of γ-glutamyl cysteine is generated from cysteine and glutamate by the enzyme glutamate cysteine ligase (GCL). The addition of glycine to the dipeptide is catalyzed by GSH synthase (GS). GSH can only be degraded extracellularly by the membrane-bound enzyme γ-glutamyl transpeptidase (GGT), generating γ-glutamyl amino acids. γ-glutamyl-amino acidsare transported via the blood stream, taken up by cells and tissues, and converted to 5-oxoproline in the reaction with γ –glutamyl cyclotransferase (GGCT). 5-Oxoproline is converted to glutamate and cysteine by 5-oxoprolinase (pyroglutamate hydrolase) with the energy input from ATP hydrolysis (Wang & Asmis 2020).

2.3.3 Chemical properties and characteristics of GSH

GSH is a linear tripeptide in which the glutamyl moiety is bound via the v-carboxyl group**(Chen *et al*.,2013).** The 't: glutamyl linkage renders the molecule resistant to normal peptidase action. The thiol moiety of the cysteinylresidue is involved in the functions of GSH as an intracellular reductant and nucleophile: it is

able to one-electron reactions including the reactions of free radicals with GSH to form the GS' free radical which dImerisizes to produce GSSG. Free radicals arise through the interaction of oxygen with certain intermediates, through biotransformation of several drugs and from ionizing radiation **(Wang *et al*.,2014;Yin *et al*.,2017).**

It is well known that oxidation caused by reactive oxygen species (ROS) is a major cause of cellular damage and death and has been implicated in cancer, neurodegenerative, and cardiovascular diseases. Small-molecule antioxidants containing sulfur and selenium can ameliorate oxidative damage, and cells employ multiple antioxidant mechanisms to prevent this cellular damage**.(Battin *et al* .,2009).**The tripeptide glutathione (y-glutamyl cysteinylglycine) has many important functions: it is an antioxidant, is involved in the detoxification of xenobiotics, and serves as a cofactor in isomerization reactions.

(Webb,2011).

The activity of GSH reductase , which requires NADPH as an energy source, ensures that most GSH is present in cells in the reduced form. Furthermore, glutathione transferases (GSTs), a large family of enzymes that covalently link reactive chemicals with GSH, aid in detoxification and excretion of toxic substances**.(Franco *et al*.,2007).**

2.3.4 Detoxifying functions of GSH

Detoxification of xenobiotics or their metabolites is one of the major functions of GSH **,(Dasari *et al* .,2018).** These compounds are electrophiles and form conjugates with GSH either spontaneously or enzymatically in reactions catalyzed by GSH S-transferase **(Potęga *al* et.,2021).** The conjugates formed are usually excreted from the cell and, in the case of hepatocytes, into bile. The metabolism of GSH conjugates begins with cleavage of the g-glutamyl moiety by GGT, leaving a cysteinyl-glycine conjugate**.(Akhavan& Anderson 2007).** The cysteinyl-glycine bond is cleaved by dipeptidase, resulting in a cysteinyl conjugate. This is followed by N-acetylation of the cysteine conjugate, forming a mercapturic acid . The metabolism of GSH conjugates to mercapturic acid begins either in the biliary tree, intestine, or kidney, but the formation of the N-acetylcysteine conjugate usually occurs in the kidney **(Sreekumar *et al* .,2021).** In addition to exogenous

compounds, many endogenously formed compounds also follow similar metabolic pathways. Some examples include estradiol-17-b, leukotrienes, and prostaglandins. Although the majority of the conjugation reactions to GSH result in detoxification of the compound, occasionally the product itself is highly reactive. One such example is the GSH conjugate of dibromoethane . GSH conjugation irreversibly consumes intracellular GSH. **(Tian *et al* .,2020).**

2.3.5 Glutathione and female infertility

Glutathione shields eggs from damage caused by oxidative stress during folliculogenesis, and as such, egg quality is dependent on it **(Adeoye *et al*.,2018).** In fact, research has shown that oocytes with higher levels of intracellular glutathione produce healthier and stronger embryos **(Hansen & Harris2015).** Another study has shown that in younger years, women's ovaries have higher intracellular glutathione levels**(Verschoor & Singh2013).**

Reduced glutathione (GSH) plays a central role in a multitude of biochemical processes, and disturbances in its homeostasis are implicated in the etiology and progression of a number of diseases**(Cacciatore *et al*.,2010).** GSH is required for proper protein and DNA synthesis, cell cycle regulation, thermo tolerance, exocrine secretion, maintenance, and regulation of the thiol-redox status of the cell**(Moreno *et al*.,2014),** protection against oxidative damage, detoxification of endogenous and exogenous reactive metals and electrophiles, biosynthesis of mercapturic acids (S-substituted N-acetyl-l-cysteines), and storage and transport of cysteine. **(Traverso *et al*.,2013).**

3. Materials and Methods

3.1. Materials

3.1.1. Instruments and Equipment:

All the device utilized as a part of this study are summered in Table 3.1.

Table 3.1. Apparatus and equipment with their manufactures.

No.	Apparatus & Equipment	Company	Manufactures
1.	Anatomical set (Scissors, Forceps,	Chemo lab	China
2.	Balance	Denver	Germany
3.	Beakers (100, 250, 500, 1000)	Chemo lab	India
4.	Centrifuge	Hettich	Germany
5.	Colony flask	Chemo lab	India
6.	Cotton	India	Entrepreneur
7.	Digital balance	Denver	Germany
8.	Digital camera	Canon	China
9.	ELIZA reader	biotek	USA
10.	ELIZA printer	epson	japan
11.	Eppendrofs tubes	Chemo lab	China
12.	Filter paper	Chemo lab	China
13.	Gel tube	Chemo lab	Chine
14.	Incubator	Lab tech	Korea
15.	Light Microscope	Human scope	Germany
16.	Microtome	Leica RM	USA
17.	Micropipettes (different volumes)	dragonmed	China
18.	Microscope with camera	MEIJI	Japan
19.	Pipette tips (10 – 1000) µl volume	Chemo lab	China
20.	Refrigerator	denka	japan
21.	Sensitive balance	Sartorius lab	Germany
22.	Slide & cover slip	Chemo lab	China
23.	Spectrophotometer	EMCLAB	Germany
24.	Surgical gloves	Chemo lab	China
25.	Syringe (1 ml, 5 ml)	Chemo lab	China
26.	Test tubes	Chemo lab	China
27.	Vortex	Sturat scientifec	United
28.	Water bath	labtech	Korea

3. 1.2. Chemicals and Kits

All the chemicals and the standard kits used in this study were shown in Table 3.2.

Table 3.2: Chemicals and Kits with their suppliers.

No.	Chemicals & Kits	Company	Suppliers
1	Absolute ethanol	Scharlau	Spain
2	Chloroform	Scharlau	Spain
4	D-galactose	Thomas Baker	India
5	Formalin 10 %	Iraqi co.	Iraq
6	Glutathione	laboratorio ct	Italy
7	(FSH) ELISA Kit	monobind	USA
8	(LH) ELIZA Kit	monobind	USA
9	Progesterone ELIZA Kit	monobind	USA
10	Estrogen ELIZA Kit	monobind	USA
11	Triglyceride	Biolabo	France
12	Xylene	Alph chemika	India
13	Paraffin wax	Citotest	China
17	High Density lipoprotein-cholesterol	Biolabo	France
18	Low Density lipoprotein cholesterol	Biolabo	France

19	D.P.X	ThomasBaker	India
20	Ethanol	Labort	India

3.2. Physical examination methods

3.2.1. Experimental protocol

Use twenty (20) adult white female rats, of which 10 males were used for insemination only, while the 20 sexually mature females were used (10 weeks of age) with a weight of 160-250 g are taken, and the rats are kept in cages with free access to water and food, and a 12/12 hour light cycle and the temperature is controlled .They were kept for 2 weeks for adaptation with standard experimental condition.

3.2.1.1.Experimental Design

Twenty (20) white female rat randomly divided into four (4) groups

(5 /group) and treated as follows for six weeks.

1. Group I: - Rats of this group were injected with only normal saline (0.1) ml serving as control ,before and during 7 day of pregnant
2. Group II: - Rats of this group were injected intra peritoneal 100mg/kg B.W of D-galactose, before and during 7 day of pregnant **(Li *et al*.,2011)**

3. Group III: - Rats of this group were injected intra peritoneal 100mg/kg B.W of glutathione, before and during 7 day of pregnant **(Ahmadvand *et al*.,2018)**

4. Group IV:- Rats of this group were injected intra peritoneal 100mg/kg B.W of glutathione and injected intra peritoneal 100mg/kg B.W of D-galactose, before and during 7 day of pregnant

3.2.1.2. The mating and timing of pregnancy

Two sexually mature females were placed with one male in each cage throughout the night, and it was confirmed that mating took place the next morning through gross examination and observation of the presence of the vaginal plug (Nau, 1992). The vaginal plug consists of a mixture of secretions of the vesicular glands and glands Coagulatory glands for the male, as this substance appears after 16-24 hours and remains about 48 hours. The percentage of dependence on this method to ensure pregnancy ranges from 80-90%, after which the females who owned the vaginal plug were isolated and the day on which they were observed is considered the zero day of Pregnancy and the next day is the first day of pregnancy (Waterman, 1976), or vaginal swabs were taken to detect the presence of male sperm in the vaginal swab, as its presence in the swab was considered a sign of pregnancy (Eveline et al., 2002). And the female that gave a positive result (the presence of male sperm in the vaginal swab) was taught and isolated in a cage alone provided with water and fodder for the purpose of conducting subsequent experiments on it.

3.2.1.3. Collect of the blood samples

Blood samples were drawn after starving the animals throughout the night

After 45 of the experiment, the animals anesthetized by chloroform Inhalation in order to control and calm the animal before the blood draw.

Seven ml blood was drawn from the heart by means of a heart puncture directly by the way the animal lay lay on its back, and sterile medical syringes of 5 ml were used, then the blood was placed In special gel tube not containing an anticoagulant, the serum was separated by a centrifuge at a speed of 3000 r / min for 15 minutes ,the separated serum put in a Eppendrofs tubes and kept in freeze at -20 ° C until the completion of the measurements.

And sacrificing pregnant females from the control and treatment groups in the 7 days of pregnancy After anesthesia, pregnant rats were placed in a dissection dish and a longitudinal incision was made with sharp scissors in their abdomen to extract the two uterine horns containing the fetus.

3.2.1.4 Organs collection for Histological section

After the end of the experiment, rat were sacrificed by anesthesia using chloroform, and the animals were dissected to remove samples (uterus and ovary) and the organs were preserved in formalin at a concentration of 10% in clean plastic containers after numbering them until Perform the Histological section.

3.3 Measurement enzyme concentration

The enzyme concentration was measured by using a special Enzyme-Linked Immunosorbent Assay (ELISA) kit, and it was measured by the steps included in the kit.

3.4. Measurement of biochemical parameters

3.4.1Estimation of Follicle Stimulating Hormone (FSH) Concentration

In the serum, the level of the hormone FSH was measured using a Kit Specific to measure the hormone level depending on the method of using Kit According to the instructions of the producing company , as shown appendix **I**.

3.4.2 Estimation of Lutanizing Hormone (LH) Concentration

In the serum, the level of the hormone LH was measured using a Kit Specific to measure the hormone level depending on the method of using Kit According to the instructions of the producing company , as shown appendix II.

3.4.3 Estimation of Hormone Progesterone in the serum

The level of the hormone Progesterone was measured using a Kit Specific to measure the hormone level depending on the method of using Kit According to the instructions of the producing company , as shown appendix III.

3.4.4Estimation of Hormone Estrogen Concentration

In the serum, the level of the hormone Estrogen was measured using a Kit Specific to measure the hormone level depending on the method of using Kit According to the instructions of the producing company , as shown appendix IV.

3.4.5. Determination of Serum Malondialdehyde (MDA) concentration (µ mol /L): Malondialdehyhe was estimated by Thiobarbituric acid (TBA) assay method of (Buege and Aust, 1978) on spectrophotometer, As Show appendix V.

3.4.6 Determination of serum Reduced Glutathione peroxidase concentration

Reduced glutathione was measured following the method of (Sedlak and Lindsay ,1968) , As Show appendix VI.

3.4.7 Determination of serum concentration of Catalyase (cat)

M. H.Hadwan and H.N. Abed . (2016). Data supporting the spectrophotometric method for the estimation of catalase activity. Data in Brief 6 :194-199, As Show appendix VII.

3.4.8 Determination of serum concentration of SOD activity determination

measured following the method of (Marklund & Marklund 1974) As Show appendix VIII.

3.4.9Determination of Serum Total Cholesterol (TC) Concentration (mg/dl):

Serum Total Cholesterol concentration has been measured enzymatically according to(Allain *et al*., 1974). show sn in appendix IX.

3.4.10 Determination of Serum Triglyceride (TAG) Concentration (mg/dl):

Serum Triglyceride concentration has been measured enzymatically by utilizing Triglyceride (TAG) kit (Table 3.2), according to (Fossati and Prencipe,1982), as illustrated in appendix X.

3.4.11 Determination of Serum High Density lipoprotein-cholesterol (HDL-C) Concentration (mg/dl):

Serum High Density lipoprotein concentration was measured enzymatically by utilizing high density lipoprotein (HDL-C) kit (Table 3.2), according to (Naito and Kaplan, 1984),as illustrated in appendix XI

3.4.12 Determination of Serum Low Density lipoprotein-cholesterol (LDL-C) Concentration (mg/dl):

Serum low density lipoprotein-cholesterol concentration was measured depending on equation (Friedewald et al., 1972), as illustrated in appendix X11.

3.4.13 Determination of Serum Very Low-density lipoprotein-cholesterol (VLDL-C) Concentration (mg/dl):

Serum very low density lipoprotein-cholesterol concentration was measured depending on equation (Friedewald et al., 1972), as illustrated in appendix XIII.

3.4.14 Statistical analysis

Statistatical analysis of data for four experiments of present study was performed on the basis of two way analysis of variance (ANOVA) using significant level of ($P<0.05$) .Differences were determined using least significant differences (LSD) (Steel and Torries, 1980)

3.6. Histological preparations

The samples (uterus and ovary) the organs were preserved in formalin at a concentration of 10% in clean plastic containers after numbering them for until Perform the Histological section.

3.6.1.Dehydration and Clearing

Water was withdrawn from the tissue by passing it through a series of ascending consternation of alcohol (70%, 80%, 90%, 95%, and 100%) for two hours for each concentration. And after that the tissue placed in xylene for 5 min .

3.6.2.Infiltration

After completion of the lamination process, the samples were transferred to glass bottles containing a mixture of Paraffin wax with a melting point of 57-60 °C, molten, filter and xylene at a ratio of 1:1 for half an hour inside an electric oven at a temperature of 60 °C in order to keep the wax melted and to ensure the complete impregnation process For wax models, they were transferred to other bottles containing paraffin wax inside the oven for two hours, then transferred again to other bottles containing paraffin wax for two hours as well.

3.6.3.Embedding

Wax molds were made to contain the samples by pouring the wax into special iron molds in which the models were buried and left at the laboratory temperature to harden, then separated from the mold and preserved until the time of cutting.

3.6.4.Sectioning

A rotary manual microtome was used to cut the models with a thickness of 5 micrometers, then the sections tapes were loaded onto clean glass slides after they were placed in a water bath at a temperature of 45-50 ° C for a period of one minute - two minutes to ensure that the sections were brushed and then left on a hot plate to dry at 37°C

3.6.5.Staining

The following special dyes were used to color the tissue sections of the different types of tissues.

3.6.5.1.Harris' Hematoxylin

The stainer was prepared according to the following steps and based on (Suvarna *et al.,* 2013), as illustrated in appendix XIV.

3.6.5.2.Eosin stain

The stainer was prepared according to the following steps and based on (Suvarna *et al*., 2013) as illustrated in appendix XV.

3.6.5.3.Gomori's One-Stap Trichrome Stain

This stain was prepared according to Hansen (2006) method, where it is used to color the colloidal fibers and smooth muscle fibers, as the collagen fibers appear in green color, while the smooth muscle fibers, the nucleus and the cytoplasm are colored red , as illustrated in appendix XVI.

Experiment design

20 female and 10 male rat sexual mature injected IP for 45 days before and during 7 days of pregnancy

5 female injected IP with normal saline G1 (control group)	5 female injected IP with D-gal (100mg /kg B.W) IP (G2 group)	5 female injected IP with GSH (100mg /kg B.W) IP (G3 group)	5 female injected IP with D-gal+ GSH (100mg /kg B.W) IP (G4 group)

At 7 Day of pregnancy, all the animals from each group were sacrificed and fallowing parameter was measured:

Hormonal analysis	Antioxidant status and Glucose level	Lipid profile	Histological study
FSH LH Estrogen progesterone	GSH,MDA,CAT And SOD	TC,TG,HDL-c,LDL-c And VLDL-c	Uterus Ovary

4.Results

4.1.1 Effect of D-galactose, glutathione and there combination on serum follicle stimulating hormone (FSH) in female rats before and during 7 day of pregnancy:

The results from the table (4-1) showed dramatically decrease in FSH level before the pregnancy period among G4 in comparisontoG1 ,G2 and G3 at a significant level of ($p<0.05$). As well as the results showed that is no difference appeared in FSH level between G2 and G3 compared to G1.

Regarding to the 7 days pregnancy period ,the data showed that the level of FSH was reduced obviously in g2 compared toG1 and G3 ,additionally G4 displayed similar results for G2, in contrast there is no significant diference among G3 in compered to G1 at level of ($p<0.05$).

However , the level of FSH was extremely lessen in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-1) Effect of D-galactose, glutathione and there combination on serum follicle stimulating hormone (FSH) in female rats before and during 7 day of pregnant.

Groups / Period	G1 control Injected with normal saline	G2 Injected with D-gal. 100mg /kg	G3 Injected with GSH 100mg /kg	G4 Injected with 100mg/kg D-gal and100mg/kg GSH.
Before pregnant	**4.07 ±0.26 Aa**	**3.11 ±0.51 Aa**	**3.35 ±0.62 Aa**	**2.65 ±0.01 Ba**
7 days of pregnancy	**3.81 ± 0.40 Aa**	**2.16 ± 0.18 Bb**	**3.53 ± 0.31 Aa**	**2.39 ±0.16 Ba**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH

4.1.2 Effect of D-galactose, glutathione and there combination on serum Luteinizing hormone (LH) in female rats before and during 7 day of pregnant

The results from the table (4-2) showed dramatically increase in LH level before the pregnancy period among G2 in comparison toG1 ,G3 and G4 at a significant level of ($p<0.05$). As well as the results showed that is no difference appeared in LH level between G3 and G4 .

Regarding to the 7 days pregnancy period ,the data showed that the level of LH was reduced obviously in G2compared toG1,G3 and G4,

However , the level of LH was extremely lessen in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-2). Effect of D-galactose, glutathione and there combination on serum Luteinizing hormone (LH) in female rats before and during 7 day of pregnant

Groups / Period	G1 Control Injected with normal saline	G2 Injected with D-gal. 100mg /kg	G3 Injected with GSH 100mg /kg	G4 Injected with 100mg/kg D-gal and100mg/kg GSH.
Before pregnant	3.01 ±0.33 Aa	3.77 ± 0.51 Aa	2.66 ±0.43 Ba	2.06 ±0.25 Ba
7 days of pregnancy	2.34 ±0.25 Ab	1.427 ± 0.10 Bb	2.60 ± 0.30 Aa	2.17 ±0.31 Aa

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.1.3 Effect of D-galactose, glutathione and there combination on serum Estrogen hormone (E2) in female rats before and during 7 day of pregnant

The results from the table (4-3) showed dramatically decrease in Estrogen level before the pregnancy period among G3 in comparison to G1 ,G2 and G3 at a significant level of ($p<0.05$). As well as the results showed that is no difference appeared in Estrogen level between G4 and G1 compared to G3.

Regarding to the 7 days pregnancy period ,the data showed that the level of Estrogen was increase obviously in G2 compared toG1,G3 and G4. in contrast there is no significant difference among G4 in compered to G1 at level of ($p<0.05$). However , the level of Estrogen was no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table 4-3 effect of D-galactose, glutathione and there combination on female rat serum estrogen hormone before and day 7 of pregnant.

Groups / period	G1 Control Injected with	G2 Injected with D-gal. 100mg /kg	G3 Injected with GSH 100mg /kg	G4 Injected with 100mg/kg D-gal

	normal saline			**and100mg/kg GSH.**
Before pregnant	**162.33 ±3.51 Aa**	**200.19 ±10.42 Ba**	**151.11 ±9.35 Ca**	**166.38 ±11.00 Aa**
7 days of pregnancy	**160.19 ±10.88 Aa**	**211.55 ± 4.40 Ba**	**149.17 ± 8.87 Aa**	**168.93 ±3.27 Aa**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.1.4 Effect of D-galactose, glutathione and there combination on serum progesterone hormone in female rats before and during 7 day of pregnant:

The results from the table (4-4) showed dramatically decrease in progesterone level before the pregnancy period among G4 in comparisontoG1 ,G2 and G3 at a significant level of ($p<0.05$). As well as the results showed that is no difference appeared in progesterone level between G3 and G1 compared to G2.

Regarding to the 7 days pregnancy period ,the data showed that the level of progesterone was reduced obviously in G2 compared toG1,G3 and G4 at level of ($p<0.05$).

However , the level of progesterone was extremely increase in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy at level of ($p<0.05$).

Table (4-4) effect of D-galactose, glutathione and there combination on female rat serum progesterone hormone before and day 7 of pregnant.

Groups	**G1 Control**	**G2 Injected with D-gal.**	**G3 Injected with GSH**	**G4 Injected with**

period	Injected with normal saline	100mg /kg	100mg /kg	100mg/kg D-gal and100mg/kg GSH.
Before pregnant	**35.61 ±1.63 Aa**	**22.59 ±2.45a Ca**	**33.41 ±1.93 Aa**	**28.11 ±2.01 Ba**
7 days after pregnancy	**50.60 ±1.19 Ab**	**33.84 ± 1.25 Bb**	**45.34 ± 4.98 Ab**	**42.39 ±5.90 Ab**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH

4.2.1 Effect of D-galactose, glutathione and there combination on serum Reduced glutathione in female rats before and during 7 day of pregnant:

The results from the table (4-5) showed dramatically decrease in GSH level before the pregnancy period among G4 in comparisontoG1 ,G2 and G3 at a significant level of ($p<0.05$). As well as the results showed that is no difference appeared in FSH level between G4 and G3 compared to G1.

Regarding to the 7 days pregnancy period ,the data showed that the level of GSH was reduced obviously in G2 compared toG1,G3 and G4 at level of ($p<0.05$).

However , the level of GSH was extremely lessen in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-5) effect of D-galactose, glutathione and there combination on female rat serum reduced glutathione before and day 7 of pregnant.

Groups	G1 Control	G2 Injected with D-gal.	G3 Injected with GSH	G4 Injected with

period	Injected with normal saline	100mg /kg	100mg /kg	100mg/kg D-gal and100mg/kg GSH.
Before pregnant	**46.11 ±0.97 Ba**	**32.05 ±1.48 Ca**	**53.12 ±1.28 Aa**	**44.27 ±1.31 Ba**
7 days of pregnancy	**42.39 ±1.17 Aa**	**28.57 ± 1.62 Bb**	**50.87 ± 2.41 Aa**	**40.34 ±2.34 Aa**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.2.2 Effect of D-galactose, glutathione and there combination on serum MDA in female rats before and during 7 day of pregnant:

The results from the table (4-6) showed dramatically increase in MDA level before the pregnancy period among G2 in comparison to G1 ,G3 and G4 at a significant level of ($p<0.05$).

Regarding to the 7 days pregnancy period ,the data showed that the level of MDA was increased obviously in G2 compared toG1,G3 and G4 at level of ($p<0.05$).

However , the level of MDA was extremely lessen in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-6) effect of D-galactose, glutathione and there combination on female rat serum MDA before and day 7 of pregnant.

Groups / period	G1 Control Injected	G2 Injected with D-gal. 100mg /kg	G3 Injected with GSH 100mg /kg	G4 Injected with 100mg/kg D-

	with normal saline			**gal and100mg/kg GSH.**
Before pregnant	**29.08 ±1.00 Ca**	**46.27 ±2.01 Aa**	**26.31 ±1.69 Ca**	**36.02 ±2.08 Ba**
7 days of pregnancy	**32.67 ±1.54 Aa**	**44. 27 ± 2.49 Ba**	**29.11 ± 1.87 Aa**	**34.67 ±5.00 Aa**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.2.3 Effect of D-galactose, glutathione and there combination on serum in Catalase female rats before and during 7 day of pregnant:

The results from the table (4-7) showed dramatically decrease in CAT level before the pregnancy period among G2 in comparison to G1 ,G3 and G4 at a significant level of ($p<0.05$).

Regarding to the 7 days pregnancy period ,the data showed that the level of CAT was reduced obviously in G2 compared toG1,G3 and G4 ,additionally no significant difference between other groups at level of ($p<0.05$).

However , the level of CAT was extremely lessen in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table(4-7) effect of D-galactose, glutathione and there combination on female rat serum catalase before and day 7 of pregnant.

Groups / period	**G1 Control Injected with**	**G2 Injected with D-gal. 100mg /kg**	**G3 Injected with GSH 100mg /kg**	**G4 Injected with 100mg/kg D-gal**

	normal saline			and100mg/kg GSH.
Before pregnant	**59.31 ±2.39 Ba**	**33.67 ±2.04 Ca**	**66.07 ±1.29 Aa**	**54.34 ±1.82 Ba**
7 days of pregnancy	**61.61 ±3.78 Aa**	**36.08 ± 3.93 Ba**	**63.70 ± 2.73 Aa**	**57.95 ±4.03 Aa**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.2.4 Effect of D-galactose, glutathione and there combination on serum in Superoxide Dismutase female rats before and during 7 day of pregnant:

The results from the table (4-8) showed dramatically decrease in SOD level before the pregnancy period among G2 in comparison to G1 ,G3 and G4 at a significant level of ($p<0.05$). As well as the results showed that is no difference appeared in SOD level between G3 and G1.

Regarding to the 7 days pregnancy period ,the data showed that the level of SOD was reduced obviously in G2 compared to G1,G3 and G4 , in contrast there is no significant difference between other groups

However , the level of SOD in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy was increased ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-8) effect of D-galactose, glutathione and there combination on female rat serum superoxide dismutase before and 7 of pregnant.

Groups / period	**G1 Control Injected with**	**G2 Injected with D-gal. 100mg /kg**	**G3 Injected with GSH 100mg /kg**	**G4 Injected with 100mg/kg D-gal**

	normal saline			and100mg/kg GSH.
Before pregnant	**2.63 ± 0.07 Aa**	**1.03 ±0.05 Ca**	**2.77 ±0.04 Aa**	**1.98 ±0.11 Ba**
7 days of pregnancy	**2.34 ±0.25 Aa**	**1.427 ± 0.10 Bb**	**2.60 ± 0.30 Aa**	**2.17 ±0.31 Ab**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.3.1 Effect of D-galactose, glutathione and there combination on serum in Total cholesterol female rats before and during 7 day of pregnant

The results from the table (4-9) showed dramatically increase in TC level before the pregnancy period among G2 in comparisontoG1 ,G3 and G4 at a significant level of ($p<0.05$).

Regarding to the 7 days pregnancy period ,the data showed that the level of TC was increased obviously in G2 compared toG1,G3 and G4 at level of ($p<0.05$).

However , the level of TC was extremely increased in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-9) effect of D-galactose, glutathione and there combination on female rat serum Total cholesterol before and 7 of pregnant.

Groups / **period**	**G1 Control Injected with normal saline**	**G2 Injected with D-gal. 100mg /kg**	**G3 Injected with GSH 100mg /kg**	**G4 Injected with 100mg/kg D-gal and100mg/kg GSH.**

Before pregnant	**260.72 ±15.51 Ca**	**370.44 ±19.42 Aa**	**251.76 ±22.35 Ca**	**300.64 ±21.00 Ba**
7 days of pregnant	**376.24 ± 13.11 Ab**	**389.78 ±12.78 Ab**	**267.12 ± 4.91 Cb**	**346.52 ±11.90 Bb**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.3.2 Effect of D-galactose, glutathione and there combination on serum in HDL-C female rats before and during 7 day of pregnant: The results from the table (4-10) showed dramatically decrease in HDL-C level before the pregnancy period among G2 in comparison to G1 ,G3 and G4 at a significant level of ($p<0.05$).

Regarding to the 7 days pregnancy period ,the data showed that the level of HDL-C was reduced obviously in G2 compared toG1,G3 and G4 at level of ($p<0.05$).

However , the level of HDL-C was extremely lessen in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-10) effect of D-galactose, glutathione and there combination on female rat serum HDL-C before and 7 of pregnant.

Groups / period	**G1 Control Injected with normal saline**	**G2 Injected with D-gal. 100mg /kg**	**G3 Injected with GSH 100mg /kg**	**G4 Injected with 100mg/kg D-gal and100mg/kg GSH.**
Before pregnant	**118.53 ±5.52**	**80.61 ±4.22**	**107.05 ±4.35**	**91.96 ±3.39**

	Aa	**Ba**	**Ca**	**Aa**
7 days of pregnancy	**95.00 ± 7.53 Ab**	**66.83 ±3.43 Cb**	**94.22 ±13.02 Ab**	**87.63 ±13.85 Bb**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.3.3 Effect of D-galactose, glutathione and there combination on serum in LDL-C female rats before and during 7 day of pregnant : The results from the table (4-11) showed dramatically increase in LDL-C level before the pregnancy period among G2 in comparison to G1 ,G3 and G4 at a significant level of ($p<0.05$).

Regarding to the 7 days pregnancy period ,the data showed that the level of LDL-C was reduced obviously in G2 compared toG1,G3 and G4 at level of ($p<0.05$).

However , the level of LDL-C was extremely increase in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-11) effect of D-galactose, glutathione and there combination on female rat serum LDL-C before and 7 of pregnant.

Groups / period	**G1 Control Injected with normal saline**	**G2 Injected with D-gal. 100mg /kg**	**G3 Injected with GSH 100mg /kg**	**G4 Injected with 100mg/kg D-gal and100mg/kg GSH.**
Before pregnant	**196.60 ±11.51 Ca**	**25623 ±9.42 Aa**	**212.45 ±10.35 Ca**	**233.06 ±10.00 Ba**

7 days of pregnancy	**246.11 ±17.28 Bb**	**283.08 ±23.35 Ab**	**236.61 ±40.61 Cb**	**259.44 ±46.94 Bb**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.3.4 Effect of D-galactose, glutathione and there combination on serum in VLDL-C female rats before and during 7 day of pregnant :

The results from the table (4-12) showed dramatically increase in V LDL-C level before the pregnancy period among G2 in comparison to G1 ,G3 and G4 at a significant level of ($p<0.05$).

Regarding to the 7 days pregnancy period ,the data showed that the level of VLDL-C was increased obviously in G2 compared toG1,G3 and G4 at level of ($p<0.05$).

However , the level of VLDL-C was extremely increase in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-12) effect of D-galactose, glutathione and there combination on female rat serum VLDL-C before and 7 of pregnant.

Groups / period	**G1 Control Injected with normal saline**	**G2 Injected with D-gal. 100mg /kg**	**G3 Injected with GSH 100mg /kg**	**G4 Injected with 100mg/kg D-gal and100mg/kg GSH.**
Before pregnant	**12.25 ±1.01 Aa**	**18.72 ±1.12 Ba**	**13.52 ±1.05 Ca**	**11.15 ±1.00 Aa**

7 days of pregnancy	**19.76 ±1.16 Ab**	**21.85 ±0.64 Bb**	**18.87 ±0.56 Ab**	**17.59 ±1.61 Cb**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.3.5 Effect of D-galactose, glutathione and there combination on serum in TAG female rats before and during 7 day of pregnant: The results from the table (4-13) showed dramatically increase in TAG level before the pregnancy period among G2 in comparison to G1 ,G3 and G4 at a significant level of ($p<0.05$).

Regarding to the 7 days pregnancy period ,the data showed that the level of TAG was increased obviously in G2 compared toG1,G3 and G4 at level of ($p<0.05$).

However , the level of VLDL-C was extremely increase in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-13) effect of D-galactose, glutathione and there combination on female rat serum TAG before and 7 of pregnant.

Groups / period	**G1 Control Injected with normal saline**	**G2 Injected with D-gal. 100mg /kg**	**G3 Injected with GSH 100mg /kg**	**G4 Injected with 100mg/kg D-gal and100mg/kg GSH.**
Before pregnant	**61.29 ±10.01 Ba**	**79.90 ±10.22 Aa**	**65.73 ±11.35 Ba**	**62.75 ±12.10 Ba**

7 days of pregnancy	**79.06 ±7.35 Ab**	**88.70 ±9.40 Bb**	**71.13 ± 5.71 Ab**	**76.32 ±8.02 Ab**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.4 Effect of D-galactose, glutathione and there combination on serum in Glucose female rats before and during 7 day of pregnant: The results from the table (4-14) showed dramatically increase in Glucose level before the pregnancy period among G2 in comparison to G1 ,G3 and G4 at a significant level of ($p<0.05$).

Regarding to the 7 days pregnancy period ,the data showed that the level of Glucose was increased obviously in G2 compared toG1,G3 and G4 at level of ($p<0.05$).

However , the level of Glucose was extremely decrease in G2 through the 7 days of the pregnancy period compered toG2 before the pregnancy ,while no statistically seemed among other groups of both before and at the 7 days of pregnancy periods

Table (4-14) effect of D-galactose , glutathione and there combination on female rat serum glucose before and during pregnant.

Groups / period	**G1 Control Injected with normal saline**	**G2 Injected with D-gal. 100mg /kg**	**G3 Injected with GSH 100mg /kg**	**G4 Injected with 100mg/kg D-gal and100mg/kg GSH.**
Before pregnant	**106.00 ± 4.12 Ba**	**147.50 ±6.04 Aa**	**99.23 ± 6.99 Ba**	**110.9 ±3.25 Ba**

7 days of pregnancy	**98.01 ±1.23 Ba**	**124.19 ±2.88 Ab**	**97.34 ±3.07 Ba**	**100.70 ±2.41 Ba**

Values are expressed as mean ± SE n=8/ group .Capital letter denote between groups difference ($p<0.05$) vs. control. Small letter denote within group difference ($p<0.05$) vs. before and after 7das of pregnant , G1(control), G2 injected 100 of D-gal., G3 Injected 100mg/kg of GSH and G4 injected 100mg/kgB.W of D-gal and 100 mg/kg BW of GSH.

4.5:The Histological Examinations.

Results of stained sections with hematoxylin-eosin stain taken from implantation sites of female rats in the control group and the group treated for the seventh day of pregnancy showed results similar to those found in normal pregnancy and with regard to the arrangement of decidual tissue. The first event was that the cells of the stromal cells of the endometrium underwent modifications to turn into decidual tissue, which is an important indicator of the success of implantation in the antimesometrial side of the uterine mesentery. Four main areas can be identified (Fig. 1-4):

• The primary decidual zone (PDZ). The cells of the decidual tissue are compact and tightly packed that surrounds the blastocyst and the endometrium. No blood vessel was observed between the cells in this region.

• Secondary decidual tissue zone (SDZ), which is the area between the primary decidual tissue and the Undifferentiated zone (UZ). One of the important things that can be observed in this region is the presence of blood vessels and the interstitial space.

The implantation zone (IZ), which is a small area surrounding the fetus and adjacent to it in the side of the endometrium against the mesentery of the uterus.

Undifferentiated zone (UZ), which is located between the secondary decidual tissue and the muscular layer of the uterus, and its cells are smaller in size compared to the primary and secondary decidual tissue layer.

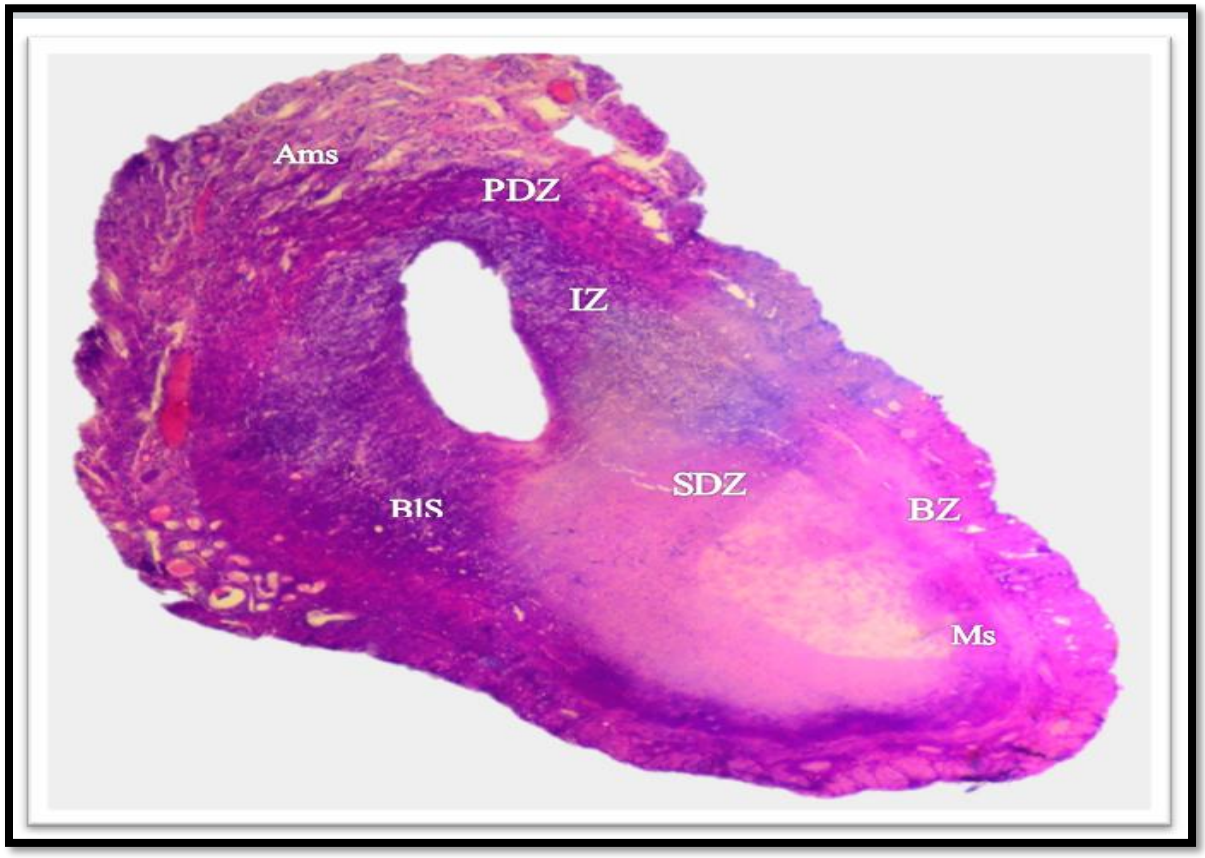


Figure (4-1) :Cross section in Rat uterus on 7 dpc (Control. subgroup = female rats were orally dosage Normal saline). Note the presence of decidual reaction. PDZ= Primary decidual zone; SDZ=secondary decidual zone; BZ= basal zone; IZ = implantation zone ; BlS= blood sinusoids of the mesometrial

decidual zone ;Ams = antimesometrial side; Ms= mesometrial side of endometrium . Hematoxylin and Eosin stain 40 X.

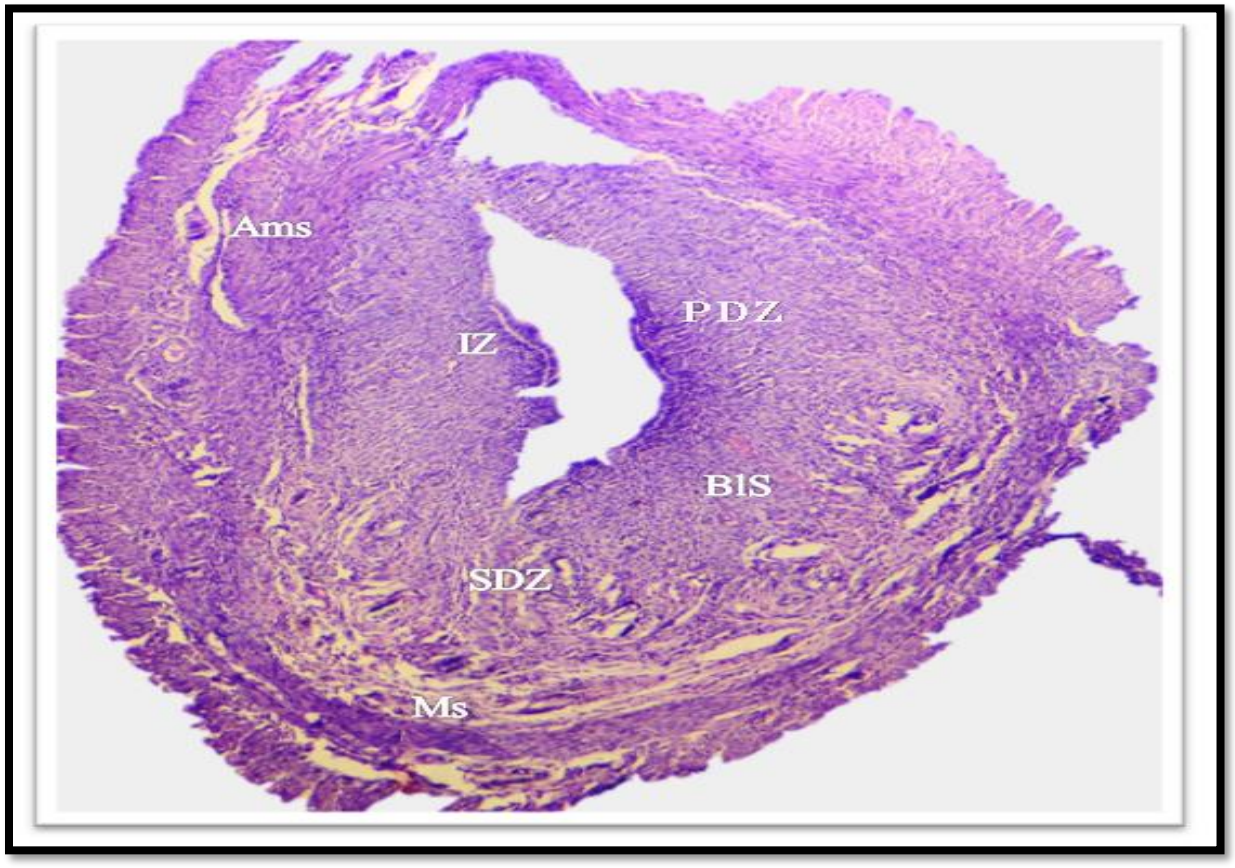


Figure (4-2) : Cross section in Rat uterus on day 7 dpc(subgroupTh. = female rats were treated with therapeutic dose of the D-galactose. Note the presence of decidual reaction. PDZ= Primary decidual zone; SDZ=secondary decidual zone; IZ = implantation zone, Ms= mesometrial side of endometrium; Am = antimesometrial side; BlS= blood sinusoids of the mesometrial decidual zone. Hematoxylin and Eosin stain 40X.

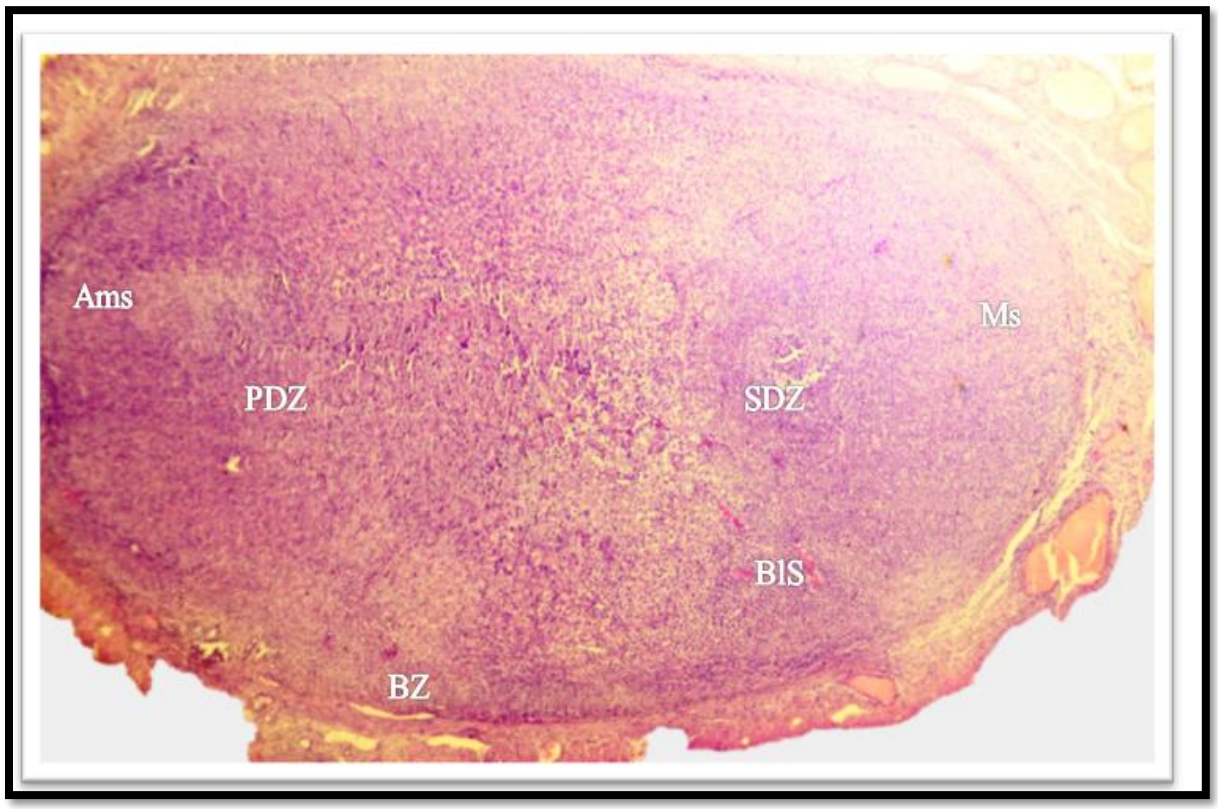
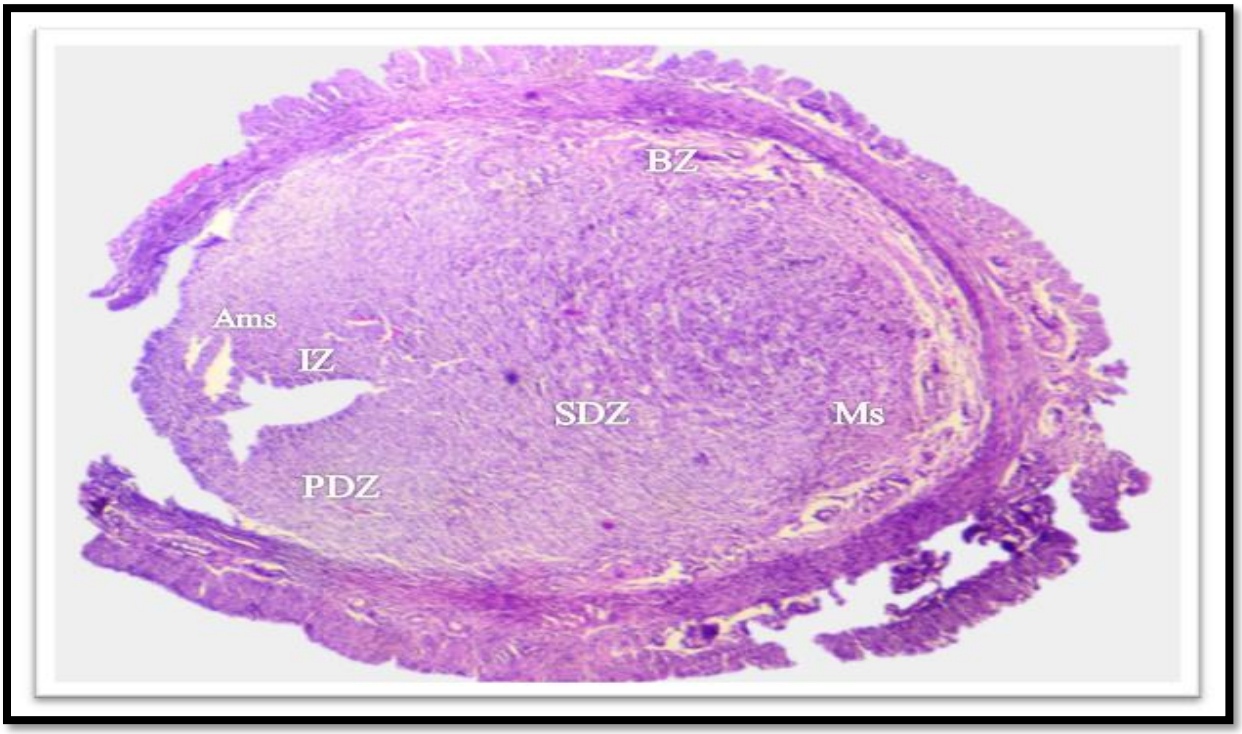


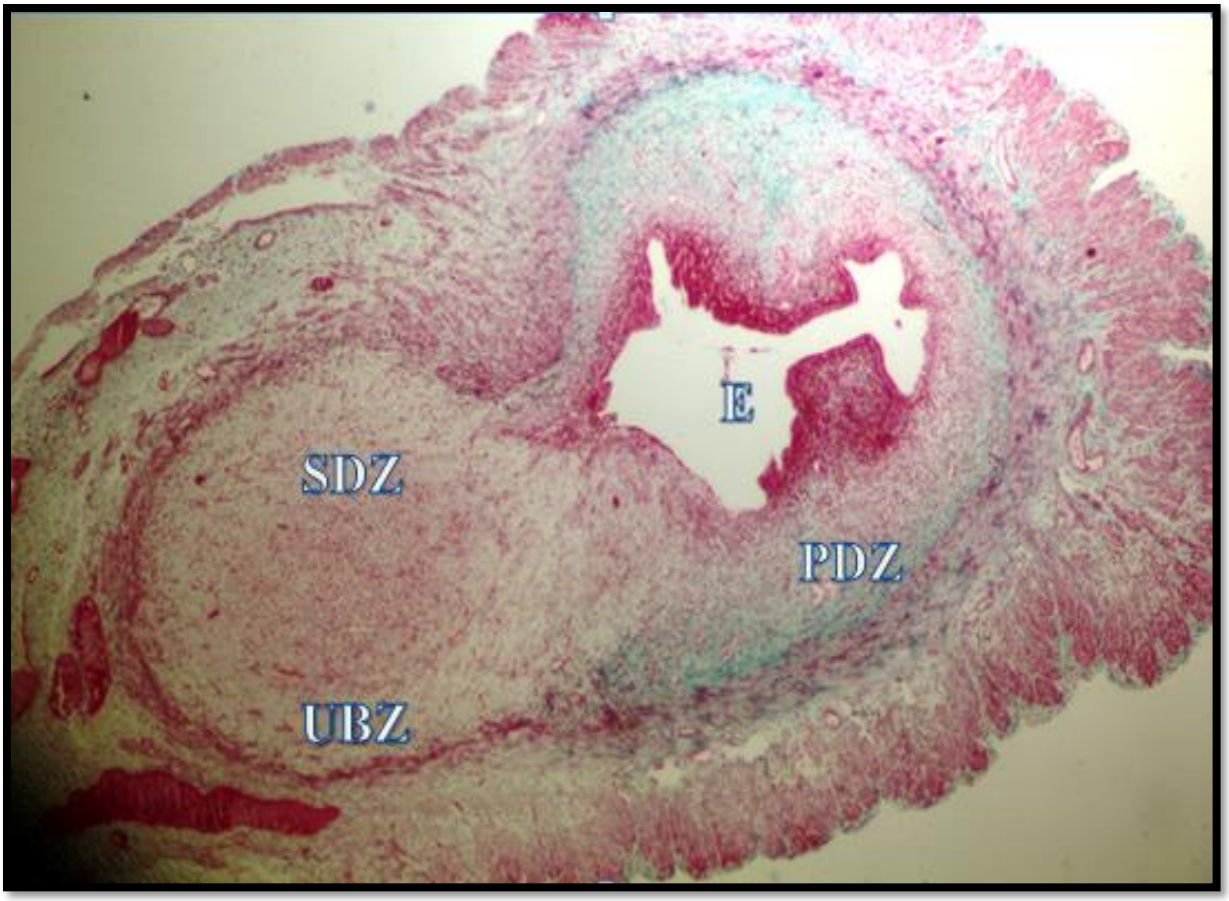
Figure (4-3): Cross section of Rat uterus on day 7 dpc (subgroups. = female rats were treated with a therapeutic dose of the GSH. Note the presence of decidual reaction. PDZ= Primary decidual zone; SDZ=secondary decidual zone; BZ= basal zone, Ms= mesometrial side of endometrium; Am = antimesometrial side; BlS= blood sinusoids of the mesometrial decidual zone. Hematoxylin and Eosin stain 40X.



Figure(4-4): Cross section of Rat uterus on day 7 dips. (Therap.subgroup, treated with D-galactose + GSH). Note the presence of decidual reaction. PDZ= Primary decidual zone; SDZ=secondary decidual zone; BZ= basal zone, IZ = implantation zone; Ms= mesometrial side of endometrium; Am = antimesometrial side; BlS= blood sinusoids of the mesometrial decidual zone. Hematoxylin and Eosin stain 40X.

As for the results of the one-step Komori multicolor colored sections taken from the implantation sites of female rats, the control group and the group that was treated with 100 mg/kg (of body weight) intraperitoneally each. From D-galactose, Glutathione, D-galactose, and Glutathione. On the seventh day of pregnancy, she showed results similar to those found in a normal pregnancy and with regard to the

arrangement of the decidual tissue. It revealed the distribution of Collagen fibers. The significant event was that the endometrium's stromal cells suffered modifications to transform into decidual tissue, which is an important indicator of successful implantation in the antimesometrial portion (photos).



Figure(4-5) :Cross section in Rat uterus on 7 dpc (Control. subgroup = female rats were orally dosage Normal saline). It is noted that there is a decidual interaction and the distribution of decidual tissue in the endometrium. It is noted that there are no Collagen fibers in the region of the primary decidual tissue and their accumulation increases as we go to the region of the secondary decidual tissue (SDZ) and from there to the undifferentiated basal region (UBZ). PDZ= Primary decidual zone. Gomori's one step trichrome stain 40 X.

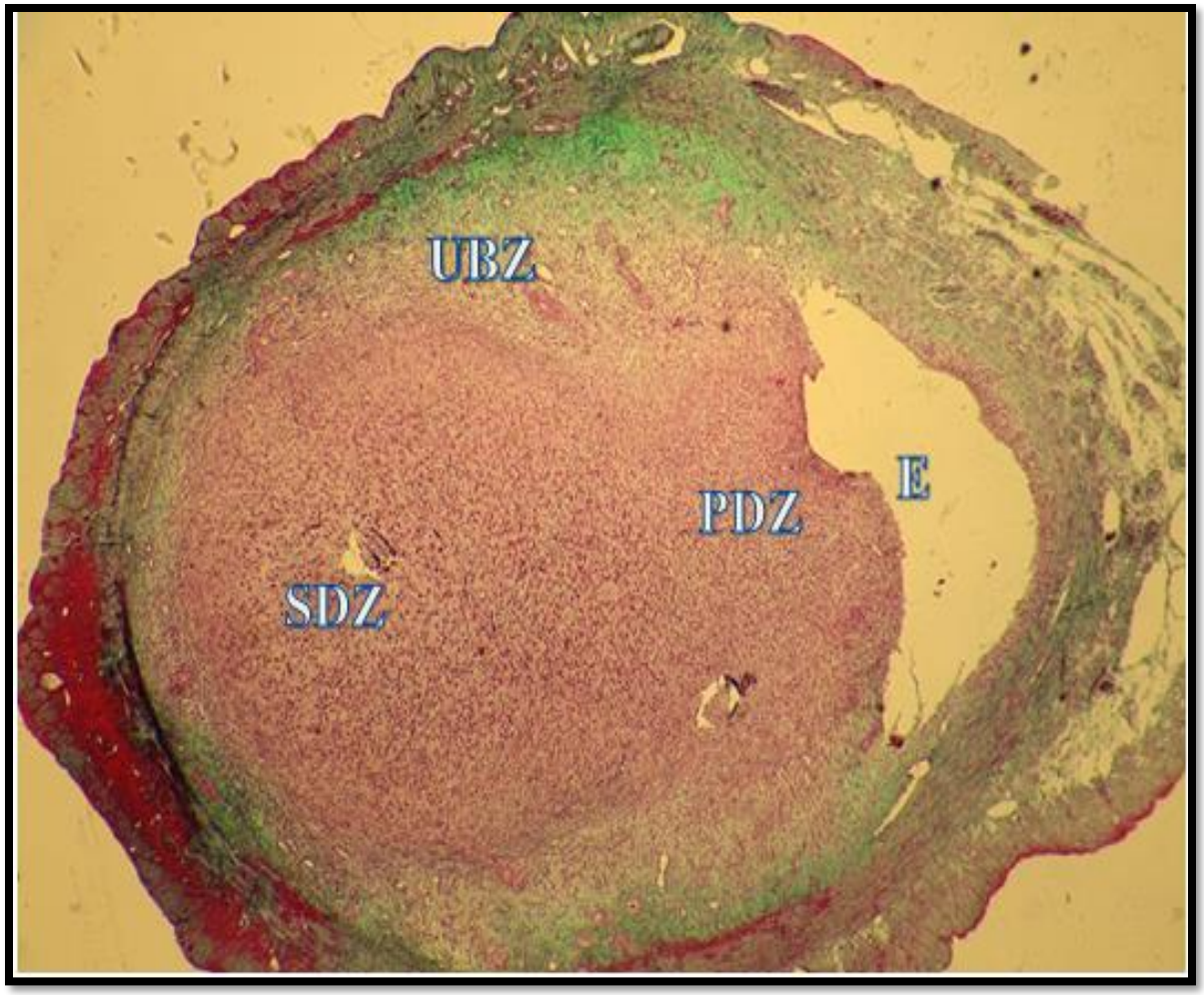
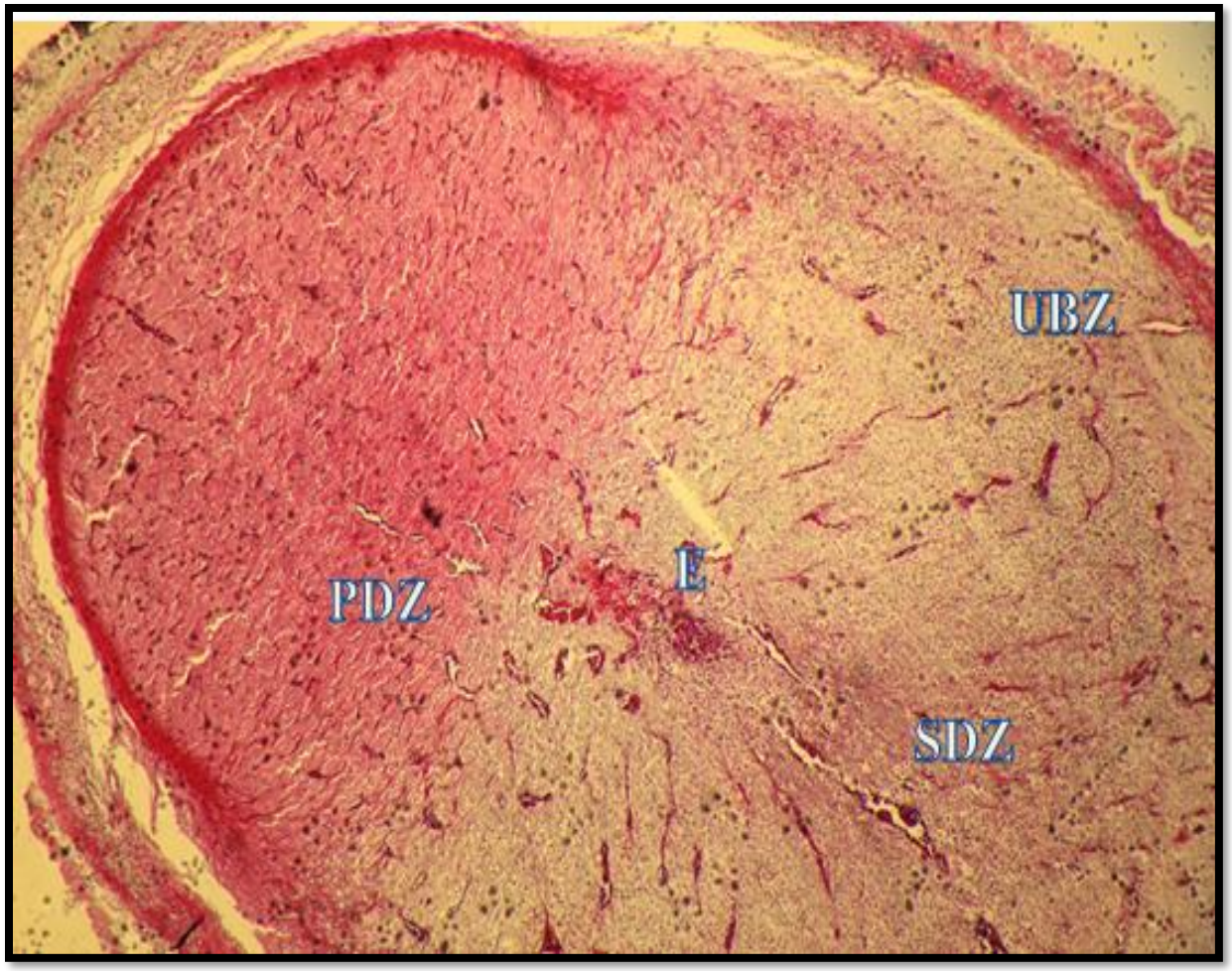


Figure (4-6) : Cross section in Rat uterus on day 7 dpc(subgroupTh. = female rats were treated with therapeutic dose of the D-galactose. It is noted that there is a decidual interaction and the distribution of decidual tissue in the endometrium. It is noted that there are no Collagen fibers in the region of the primary decidual tissue and their accumulation increases as we go to the region of the secondary decidual tissue (SDZ) and from there to the undifferentiated basal region (UBZ). PDZ= Primary decidual zone. Gomori's one step trichrome stain 40 X.



Figure(4-7) : Cross section of Rat uterus on day 7 dpc (subgroups. = female rats were treated with a therapeutic dose of the GSH. It is noted that there is a decidual interaction and the distribution of decidual tissue in the endometrium. It is noted that there are no Collagen fibers in the region of the primary decidual tissue and their accumulation increases as we go to the region of the secondary decidual tissue (SDZ) and from there to the undifferentiated basal region (UBZ). PDZ= Primary decidual zone. Gomori's one step trichrome stain 40 X.

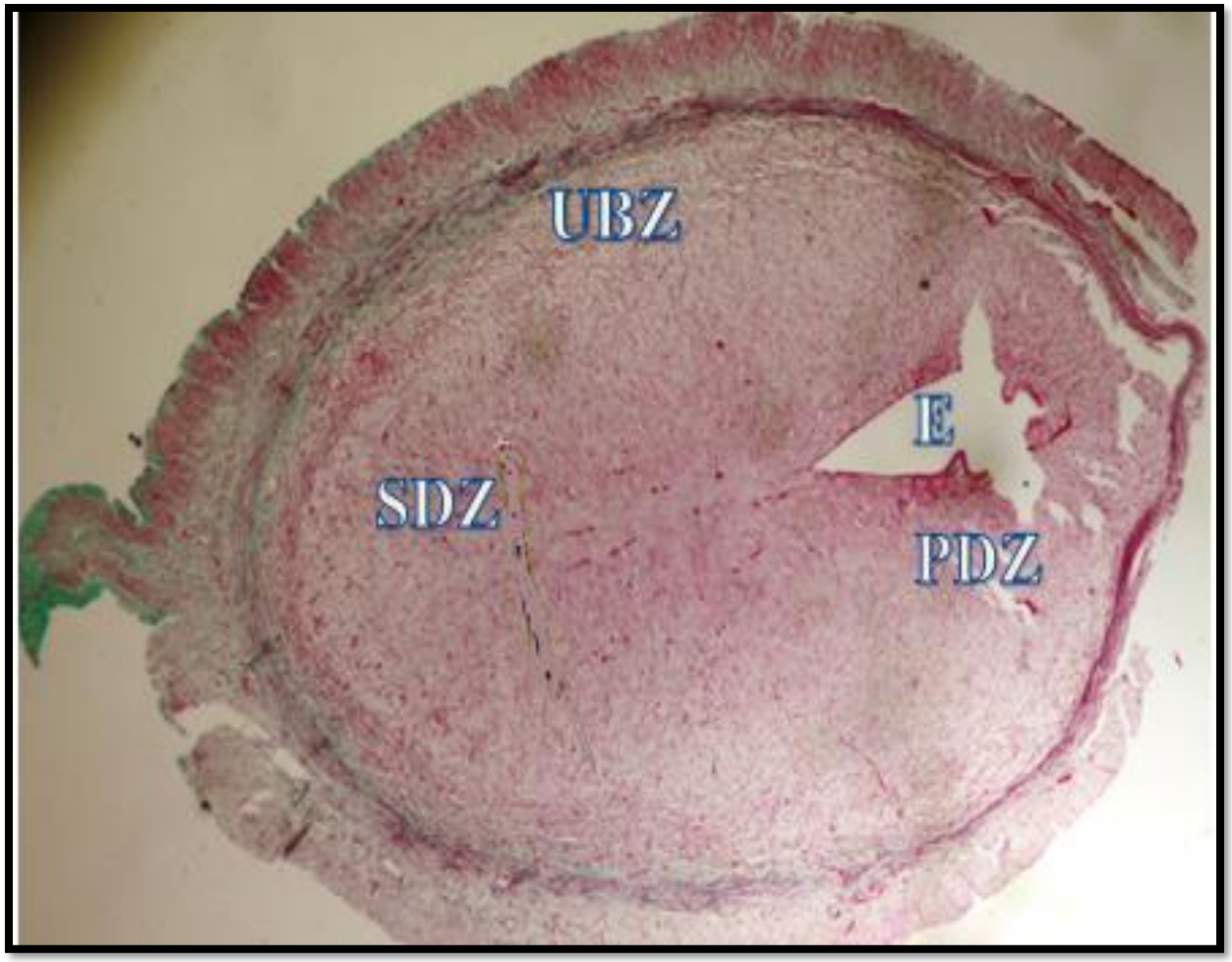
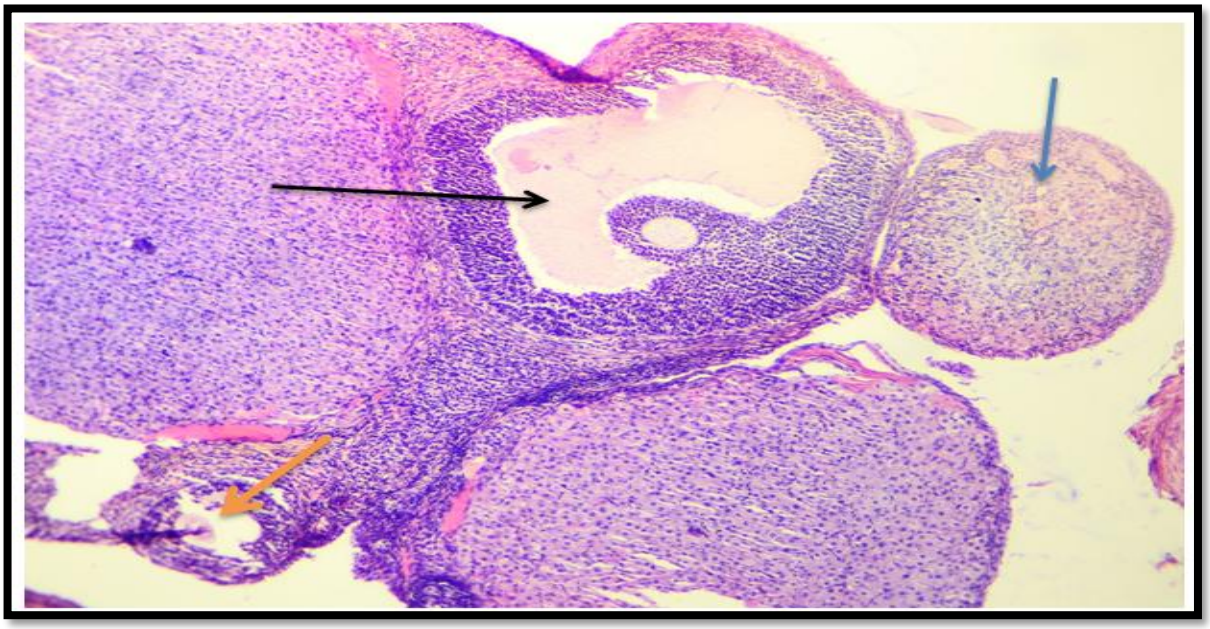


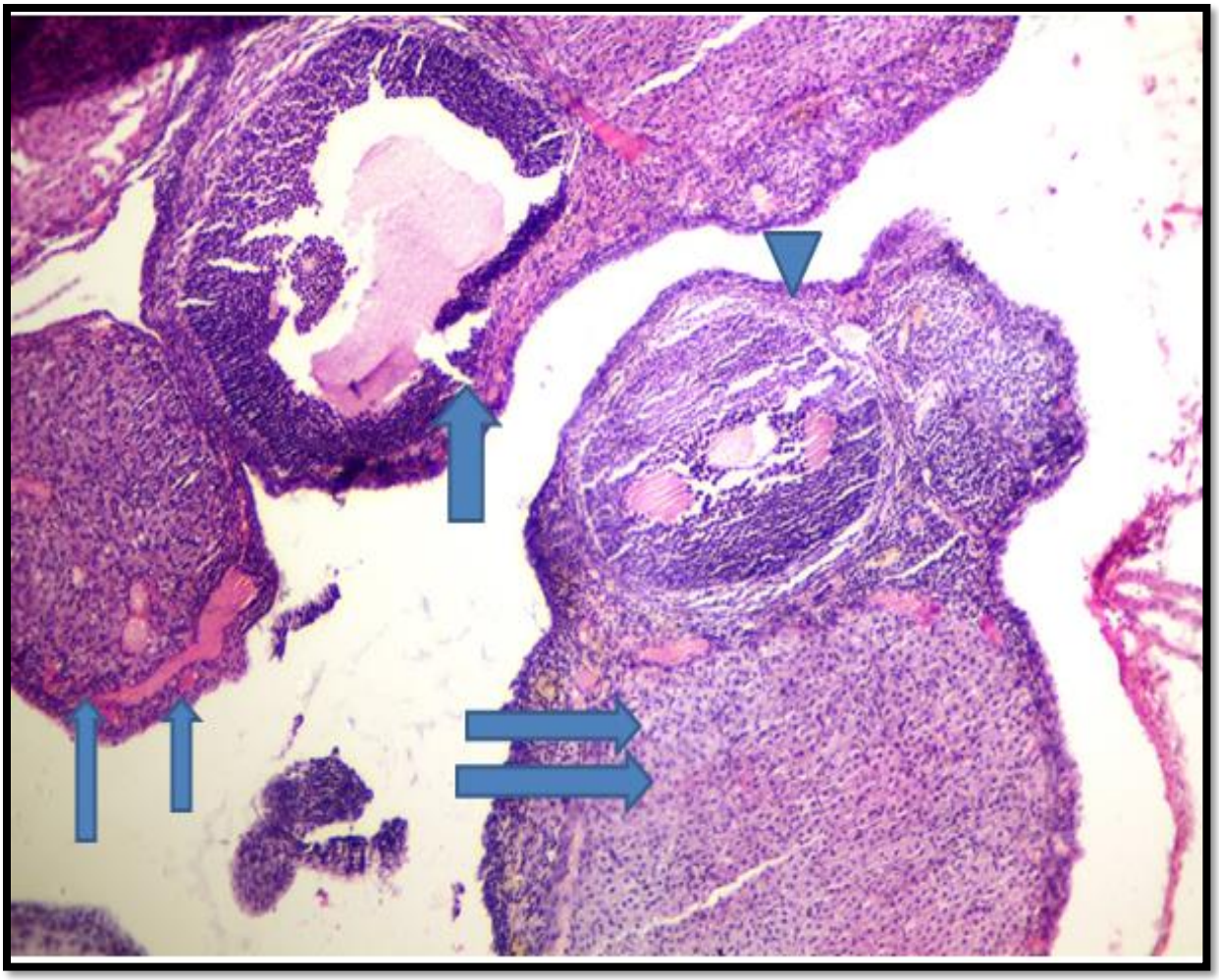
Figure (4-8) : Cross section of Rat uterus on day 7 dips. (Therap.subgroup, treated with D-galactose + GSH). It is noted that there is a decidual interaction and the distribution of decidual tissue in the endometrium. It is noted that there are no Collagen fibers in the region of the primary decidual tissue and their accumulation increases as we go to the region of the secondary decidual tissue (SDZ) and from there to the undifferentiated basal region (UBZ). PDZ= Primary decidual zone . Gomori's one step Trichrome stain 40 X.

The ovary of the rat appears in the control case, consisting of the outer cortex area, which contains ovarian follicles, and in different stages of development, such as primordial follicles, primary follicles, mature follicles, in which the atrium and the crown appear corona radiator, the granulosa membrane, and the oocyte. The follicles are located in the basic tissue of the ovary called the ovarian stroma, which consists of a network of connective tissue fibers and fibroblast-generating cells. As for the free surface of the cortex layer, it is called the surface epithelium, which is in the form of a layer of cuboidal cells, immediately below them there is a layer of dense connective tissue.

As for the inner region, it is called the medulla, which consists of loose fibroelastic connective tissue that contains a number of blood vessels**.**



Figure(4-9) :- Section in ovary of control group revealed a number of primary follicles (yellow arrow) and mature follicle (black arrow) and corpus luteum (blue arrow) (H&E stain, 100X).



Figure(4-10) :- Section in ovary of D-galactose group .The section is showing part of the mature follicle in the ovary (arrow) and part of the pregnant uterus (double arrows) . Secondary follicle in the right ovary (arrowhead) . The uterus at this stage of pregnancy is showing normal decidual reaction in the endometrium. (H&E stain, 100X).

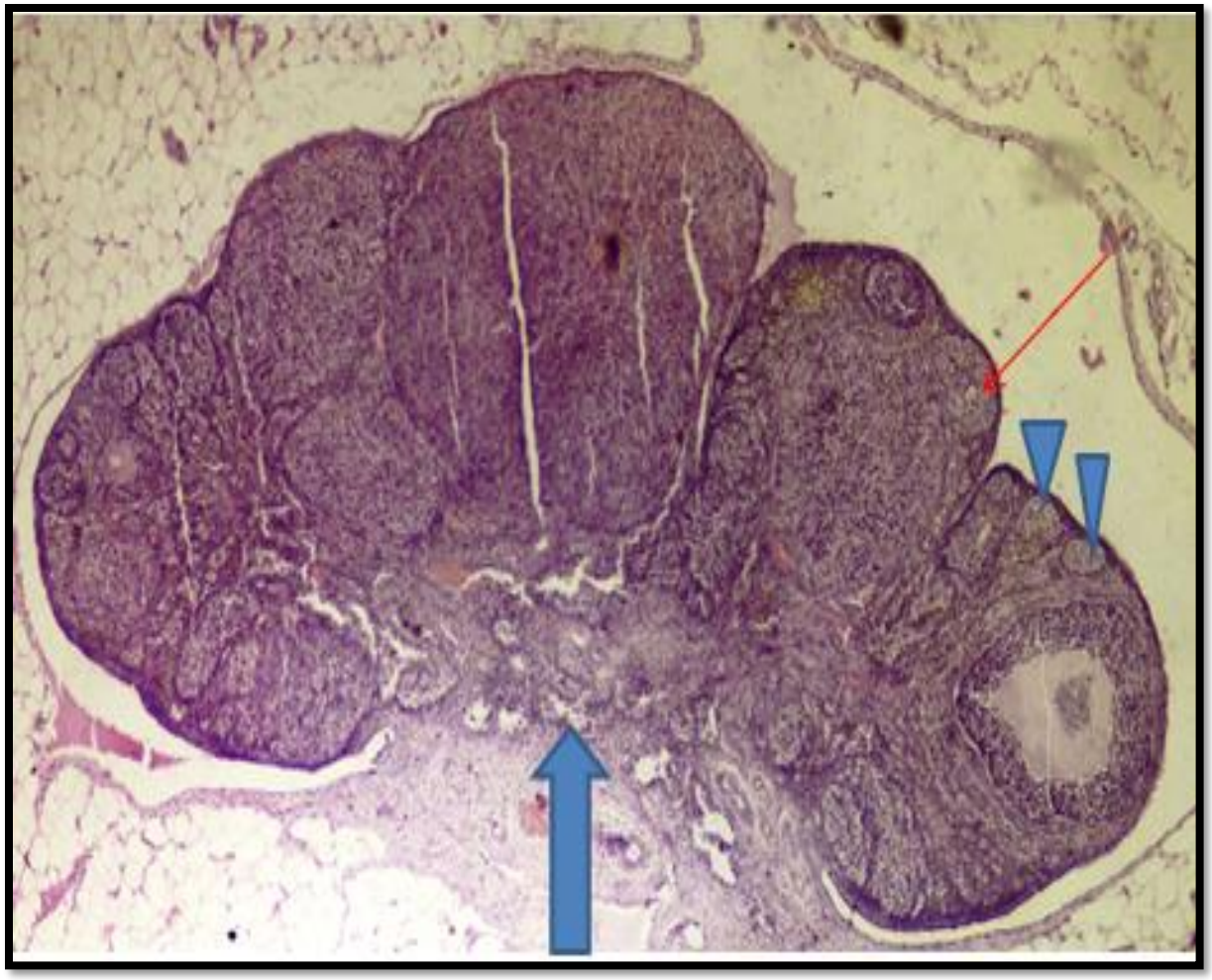


Figure (4-11) :- Section in ovary of GSH group. Section through the ovary showing different stages of ovarian follicle development in the ovarian cortex, ranging from primordial follicles (arrowheads);primary follicle(thin arrow). Highly vascularized ovarian medulla is the middle part of section (arrow) (H&E stain, 40X).

Figure (4-12) :- Section in ovary of D-galactose and GSH group revealed number of primary follicles (yellow arrow) and secondary follicle (green arrow) and corpus luteum (blue arrow) (H&E stain, 40X).

5.Discussion:

In this study, twenty sexually adult white rats were used and given D-galactose to generate oxidative stress and know its effect on implantation of the fertilized egg and study the protective effect of Glutathione.

5.1. Hormonal parameters

5.1.1Effect of D-galactose, glutathione and there combination on female rat hormone FSH and LH before and at 7 day of pregnant: from table (4-1) before pregnant D-galactose and glutathione combination group showed a significant decrease in FSH this result agreement with **(Onclin *et al*.,2002; Thakur *et al*.,2017; Salama *et al*.,2021).**

Also in the present study show signifigent decrees in FSH at 7 day of pregnancy in D-galactose group when compared to control group this result agreement with some studies that use D-galactose to induce aging and infertility in female rats **(He *et al*.,2017; Wang *et al*., 2019; Liang *et al*.,2020).**

administration of D-galactose to mammals effects on the female reproductive system, causing aging features such as endometrial atrophy, follicle degeneration and the decreased estrogen levels through modifications of oxidative stress ,this decrease in the hormone in this study is consistent with our current research**(Azman & Zakaria 2019; Zhu *et al*.,2020).**

FSH is necessary for follicle development and it is proposed that an FSH threshold should be required to obtain ovulation**(Mayorga *et al*.,2000).** Ovulation increases with increasing FSH in transgenic mice with FSH levels that increase with age, regardless of follicle depletion **(Fan *et al*.,2008).**The relationship between FSH, LH and fertility during a woman's lifetime may differ from the age-related changes of FSH and LH around menopause**.(Ruth *et al*.,2016).**

The pituitary gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are the main regulators of gonadal function, and have been extensively studied**(Nett et al.,2002;Kobayashi et al.,2010).**

Administration of D-Galactose may disrupt the estrous cycle and cause tissue damage to the uterus and ovaries **(Ahangarpour et al., 2016)**, there was a decrease

in the thickness of the endometrium and endometrial glands, and the endometrial wall was incomplete **(Saribas et al.,2020)** .During ovulation, several hormones such as gonadotropin-releasing hormone (GnRH), FSH (follicle-stimulating hormone), LH (luteinizing hormone), estrogen, and progesterone play fundamental roles in the ovulation process **(Christensen et al.,2012).**

In the present study From The main value of serum LH there is significant decrease between all group the result of the present study was agreement with **(Chen *et al* .,1984;Wunder *et al*.,2008; Piouka *et al*.,2009).** many others study the luteinizing hormone(LH) and its effect on ovarian hormones, ovulation, and progesterone formation during pregnancy **(Micevych *et al*.,2003; Stevenson & Pulley 2016;La Marca & Capuzzo 2019).** According to several studies , They provided rat during pregnancy of high galactose diet is dramatically reduced in the number of oocytes .This indicates a decrease in the luteinizing hormone **(Fridovich-Keil et al.,2011;Yu *et al*.,2011; El Bakly *et al*.,2020).**

LH acts on mature Graffian follicles to induce ovulation and luteinization of theca and granulosa cells that form the CL **(Martinez-Chequer *et al*.,2003).**

The luteinizing hormone participates in testicular and ovarian regulation performs a critical role in follicular maturation **(Vaccari *et al*.,2009),** ovulation **(Ge, 2005),** corpus luteum development and maintenance **(Niswender *et al*., 2000),** Some studies have used galactose to induce POI in laboratory animals **(Dovom *et al*.,2020).** A systematic review in 2019 showed that using different doses of galactose during the prenatal or postnatal period produced different ovarian follicle deficiency outcomes in rodents **(Sozen *et al*.,2019;Hagen-Lillevik *et al*.,2021)**.as some researchers have mentioned the Exposure to galactose during the prenatal period decreases the count of small ovarian follicles (primordial and primary) **(Hagen-Lillevik *et al*.,2021; Zhang *et al*.,2021).**

In the normal condition ROS is generated in the ovary due to increased metabolism during the final stages of folliculogenesis and follicular rupture, which results in the accumulation of ROS level possible due to decreased enzymatic antioxidants activity **(Tiwari *et al*.,2016;Wang *et al*.,2017).**

The pituitary gonadotropin hormones, LH and follicle-stimulating hormone (FSH), are the primary endocrine factors regulating apoptosis in ovarian follicles both in

vivo and in vitro**(Tsai-Turton & Luderer 2006;Saraiva *et al*.,2011).**The anti-apoptotic effects of gonadotropins appear to be mediated in part by inhibition of the mitochondrial or damage-induced apoptotic pathway **(Moindjie *et al*.,2016).** On the glutathione and D-galactose groups, glutathione's protective impact was evident on FSH and LH hormones in our study

5.1.2Effect of D-galactose, glutathione and there combination on female rat hormone Estrogen and progesterone before and at 7 day of pregnant:

From The main value of serum Estrogen there is significant decrease($p < 0.05$) in serum estrogen in GSH group when compared to control.The decrease in estrogen in the galactose group in this study is consistent with .In some studies, researchers confirmed that giving galactose to rats by Intraperitoneal injection in high amounts to stimulate oxidative stress, and it was noted that its effect is not limited only to a specific organ. But it was found that the ovary is one of the organs very sensitive to oxidative stress, and this affects the number of follicles and their maturation. Therefore, many studies have been done for women exposed to galactose toxicity. **(Lai *et al*.,2009; Fridovich-Keil *et al*.,2011; Banerjee *et al*.,2012;Yan *et al*.,2018).**

The progesterone and estrogen responsive signaling pathways integral for early pregnancy success**(Hua *et al*.,2007;Gok *et al* .,2015).** D-Galactose administration produces aging like changes such as decreased in estrogen and progesterone levels and increased in FSH and LH levels, comparable to the naturally aging control **(Ahangarpour *et al*., 2016),** and it is can induce aging because of an increase in the MDA content and decrease in the activities of SOD, catalase and glutathione peroxidase, and decrease in the estradiol content in the ovaries **(Muthusami *et al*.,2005;Kheradmand *et al*.,2010).**

Estrogens produced by the developing ovarian follicles interact with progesterone produced by the CL to prepare uterine receptivity and embryo implantation**(Young, 2013).** The CL is essential throughout pregnancy in the

mouse, rat, hamster, rabbit, dog, sow and goat, but for less than full term in cat, guinea pig, sheep, cow, horse, macaque and human**(Al-Gubory et al.,2010),** mammals may be spontaneous ovulators (e.g. ruminants, pigs, horses, and primates), induced ovulators (e.g., rabbit and cat) or spontaneous ovulators with induced CL (mice and rats).**(Bazer & Spencer 2011).**

The corpus luteum (CL) and the uterus exhibit extremely rapid cellular proliferation, growth and development to ensure hormonal environment suitable for early embryonic development and the establishment of pregnancy **(Bazer & Spencer 2011).** The establishment of pregnancy requires a receptive uterus able to respond to a variety of biochemical and molecular signals produced by the developing concepts **(Spencer & Bazer 2004).** (embryo and extra-embryonic membranes), as well as specific interactions between the uterine endometrium and the extra-embryonic membranes, the trophectoderm**(Imakawa et al.,2002).**

From The main value of serum Progesterone there significant decrease (p-value< 0.04) when compared control grope this result correlated with **(Ahangarpour *et al*.,2018; Wang *et al*.,2019; Hussein Almurumudhe & Hussein 2021).**In the present study the Decreased progesterone hormone during pregnancy is an indication of the oxidative effect of galactose administration this result correlated with **(Mohammad et al.,2012;Thakur et al.,2018;Li *et al*.,2019).**

Some scientists have proven that giving D- galactose causes an imbalance in the pituitary hormones that control the female system in the mammal , as it is highly sensitive to oxidative stress**.(Strauss & Williams 2019;Mehdi *et al*.,2021; Pérez-Torres et al.,2021).** Perhaps this decrease in the hormone is due to a defect in the corpus luteum and its insufficient progesterone secretion to maintain pregnancy. Because some research that studied the effect of galactose on women that who continuously take lactose from cow's milk will cause polycystic ovary syndrome (PCOs) and early infertility**(Dunne, 2006 ;Afeiche et al.,2016;Wright,2017).**

On the seventh day of pregnancy, in rats treated with D-galactose , a significant decrease in the level of progesterone was observed. This indicates a possible risk of miscarriage in the advanced stages of pregnancy This outcome was linked to **(De Muro *et al*., 2009;Rawlings, 2020;Fujii *et a*l.,2010).**

Progesterone is known as is a hormone generated by the corpus luteum of the follicle during the second part of the menstrual cycle that aids in the transformation and preparation of the mucosa, as well as the implantation of the fertilized egg. **(Barbu *et al*.,2021).**The uterus is the most important target organ of progesterone, which transforms the uterine lining into a tissue rich in glands, and will promote intrauterine implantation of the fertilized egg. **(Kitazawa et al.,2020).** During pregnancy, progesterone inhibits the contractions of the myometrium, but also decreases the sensitivity to physiological stimuli of contraction**(Koutras *et al*.,2021)**

5.2 Antioxidant parameters

5.2.1Effect of D-galactose and GSH on the glutathione peroxidase in serum of female rat before and at 7 day of pregnant:

Our result revealed to a significant decrease in G2 group injected(IP) with 100mg/kg of D-galactose compering with other groups before and after 7 day of pregnant while showed a significant increase in group G3 ,G4 comparing with other groups, this result agreement with **(Zhao *et al*., 2012; Garg *et al*.,2017;Rusu *et al*.,2020).**

Studies recognized that D-galactose caused significantly increased the levels of ROS during pregnancy that causes significant increase in serum GSH **(Jeremy *et al*.,2019;Bjørklund *et al*.,2021).** May be the increase in the proportion of glutathione peroxidase in the group treated with the substance that stimulates oxidative stress, which is galactose, is a result of an increase ROS in the serum , under oxidative stress, the cellular antioxidants capacity is not counterbalancing the oxidative damage induced by various insults including, free radicals and environmental toxins **(Waly *et al* .,2011).**

Antioxidant enzymes, SOD, CAT and G-Prx, are thought to be effective for augmentation of antioxidant defenses. SOD can convert superoxide radicals to hydrogen peroxide and subsequently convert to water by CAT and G-Prx **(Hou *et al*.,2004; Das & Chainy 2004; Wang *et al*.,2007)** .

D-gal is a normal nutrient that naturally exists in the body **(Kong *et al*.,2018).** As a certain dose of D-gal is injected into the rodents within a period of time, the

concentration of it in cells will be too high to be catalyzed by galactose oxidase to aldose and hydrogen peroxide, finally generating superoxide anions **(Bai *et al* .,2017).**The oxidation in the body produces a large number of free radicals, which are beyond the body's scavenging capacity and lead to lipid peroxidation; **(Lobo *et al* .,2010).** meanwhile, the final decomposition products (such as MDA) can directly or indirectly combine with proteins, nucleic acids, phospholipids, and other substances, not only destroying the chemical structure of intracellular life substances and disrupting cell function**(Kong *et al* .,2018),** but also damaging normal tissue cells, as well as affecting the normal osmotic pressure, which further lead to metabolic disorders of vital organs and eventually **(Mu *et al* .,2017).** The pathophysiological changes during natural and D-gal induced aging are related to the production of ROS and oxidative stress and it is obvious that rats of different age react differently to D-gal treatment **(Tavanai & Mohammadkhani 2017).** The pro-oxidant/antioxidant balance is successfully represented and can be assessed by the antioxidant enzyme activities and the level of lipid peroxidation **(Correia *et al*.,2003).**

Some researchers have proven that giving D-galactose induced different organ apoptosis has been documented by **(Aydın *et al*.,2018;Sun *et al*., 2020).**To minimize OS, antioxidants are useful since they scavenge free radicals and reduce ROS level in the body.

Glutathione (GSH) is present in most mammalian cells and plays an important role in cellular defense against oxidative stress by reducing protein disulfides and other cellular molecules. It also acts as a scavenger of free radicals of ROS **(Aprioku , 2013;Chevallier *et al*.,2020).,** GSH is synthesized intracellularly by two GSH-synthesizing enzymes, g-glutamylcysteine synthetase (g-GCS) and glutathione synthetase. g-GCS catalyzes the rate-limiting step of GSH synthesis . **(Ojaimi *et al* .,2010).**

In many cells, the GSH redox cycle is catalyzed by both glutathione peroxidase and glutathione reductase **(Couto & Barber 2016).** During oxidative stress, the reduced form of GSH is converted by glutathione peroxidase to oxidized glutathione **(Aksoy et al .,2005).**

In fact, research has shown that oocytes with higher levels of intracellular glutathione produce healthier and stronger embryos **(Dumollard *et al*,.2009, May)**Another study has shown that in younger years, women's ovaries have higher intracellular glutathione levels **(Chakraborty *et al* .,2013).**

It has been reported that glutathione deficiency is related to premature ovarian aging and even ovarian cancer **(Vabre *et al* .,2017).** Another study found that for women with high level of glutathione in a woman's follicle translated into increased fertilization rates**(Ebisch *et al* .,2007).**

The use of antioxidants The fertilization and embryo development in vivo takes place in an environment of low oxygen tension **(Burton et al., 2003).** Lowering oxygen tension in the culture environment also improves the implantation and pregnancy rate **(Ma *et al* .,2017).**Similarly, higher implantation and clinical pregnancy rates are reported when culture media are supplemented with antioxidants **(Agarwal et al.,2006).** GSH is directly or indirectly involved in many life activities of microbial cells. One of the most important roles of GSH is to build a strong defense line against oxygen stress together with related metabolic enzymes. GSH reduction is composed of gamma-glutamate-cysteine ligase (GSH1), glutathione synthetase (GSH2), glutathione reductase (GR), glutathione peroxidase (GPx), and NADPH **(Ighodaro & Akinloye 2018)**

5.2.2Effect of D-galactose and GSH on the MDA in serum of female rat before and at 7 day of pregnant:

Significant increase in G2 group before and during pregnant groups compering with other groups and without significant differences within groups during pregnant .The present result agreement with **(Li *et al*.,2016; Garg *et al*.,2017 ;Jeremy& Roy 2019).**

MDA is an end product of lipid peroxidation that belongs to the compounds reacting with Thiobarbituric acid. MDA diffuse to distant cellular structures where it can cause further cellular damage, including DNA damage, MDA level was used as a marker of lipid peroxidation **(Hosseini et al., 2011;Al-Beer,2013).** Lipid peroxidation is an oxidative process that normally occurs at low levels in all cells and tissues. Under normal conditions, a variety of antioxidant mechanisms serve to control this per oxidative process **(Patil *et al* ., 2009).**MDA is a lipid peroxide

formed by oxidation. The level of MDA in vivo also directly reflects the degree of oxidation **(Hosen *et al*., 2015).**

In the present study, MDA caused a decrease in the activities of SOD, GSH-Px, and CAT in serum . Some study have indicated that superoxide radicals can inhibit CAT activity and the increased H2O2 resulting from CAT inhibition could finally inhibit SOD activity**(Altuntas *et al*.,2002).**

The increase of MDA level may be due to an increase in the production of free radicals more than ability of the scavenging system to remove them increased serum MDA and decrease GSH levels ,this findings is in agreement with many laboratory studies which indicated alteration in the antioxidants status of different tissue as a results of an increase in lipid peroxidation of these tissue after induction oxidative stress **(AL-Zubaidi , 2007; Albazi,2009;Al-Okaily etal.,2015).**

Many anti-oxidant defense systems consisting of enzymatic [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] and non-enzymatic (ascorbic acid, glutathione and αtocopherol) compounds can maintain the balance between ROS generation and protection from damage by ROS **(Small et al .,2012).**

The fact that the increase in MDA levels in mature and aged D-gal treated rats was followed by an unchanged GPx activity, leads to a reasoning that either the response of GPx to increased pro-oxidant conditions is limited or the lack of change in GPx activity is a reason for increased MDA levels. **(Hadzi-Petrushev *et al*.,2015).**

Malondialdehyde is a measure of lipid peroxidation in the tissues, which considered as one of the important markers of oxidative stress that affect different organs **(Liu *et al*.,2000).** Its elevation by D-galactose in current study indicated oxidative damage induced by D-gal. Several studies suggest a strong correlation between mitochondrial damage and ROS (mainly H_2O_2) production in cells **(Chairuangkitti *et al.*, 2013;Yu *et al*.,2015).** Redox-active metals also play an important role catalyzing lipid oxidation in biological systems **(Jomova *et al*.,2012)** and thus increased level of ROS could be accompanied with elevated MDA concentration and intense depletion **(Gunawardena *et al*.,2019)**.

Although D-galactose can be changed into glucose at normal concentrations **(Kenawy *et al*.,2017; Mirshafa *et al*.,2020).**

Excessive ROS that could be produce by D-gal has detrimental effect on body organ including LPO and DNA damage **(Yuan *et al*.,2019).** In support of this finding, decreased levels of endogenous antioxidant enzymes such as SOD and Gsh-Px **(Zhang *et al*.,2006).** and reduced GSH levels **(Li *et al*.,2016),** were demonstrated in D-galactose-treated rats. Evidence shows that D-galactose administration increased expression of oxidative stress and decreased expression of antioxidants**(Rehman *et al*.,2017).**

Intracellular ROS production and propagation are controlled by highly complex and integrated antioxidant systems. Mammalian cells have evolved a variety of interrelated enzymatic antioxidant mechanisms which enable them to cope with oxidative environments **(Al-Gubory *et al*.,2010).** Besides enzymatic systems, the thiol tripeptide glutathione (GSH) is considered to be pivotal in protecting cells from ROS-induced oxidative damage **(Ercal *et al*.,2001).** Cells within the body are also protected from ROS-induced oxidative damage by various non-enzymatic dietary antioxidants **(Mankowski *et al*.,2015).**

5.2.3 Effect of D-galactose and GSH on the CAT and SOD in serum of female rat before and at 7 day of pregnant: Before pregnant in the table (4-7) showed a significant decrease ($p \leq 0.05$) in G2 compared with other groups .The mean value of serum cat show significant decrease ($p \leq 0.05$) in G2 group when comparing with other groups . In G2 at day 7 of pregnancy showed a significant increase when compared with G2 before pregnant. This result agreement with **(Vora *et al*.,2009; Haider *et al*.,2015; Hamza *et al*.,2021).**

Some researchers explained that increase in D-galactose significantly increased the levels of ROS and malondialdehyde (MDA) and decrease the activities of antioxidant enzymes such as SOD, catalase (CAT) and glutathione (GSH)

(Ho *et al*.,2003; Hadzi-Petrushev *et al*.,2015; Zeng *et al*.,2020).

To minimize OS, antioxidants are useful since they scavenge free radicals and reduce ROS level in the body**(Uttara *et al*.,2009).**Free radicals such as superoxide (O2 −), hydrogen peroxide (H2 O2) and nitric oxide are molecules that occur

during several physiological and pathological processes**(Klings & Farber 2001).** These free radicals are scavenged continuously by antioxidant enzymes such as SOD, CAT, glutathione peroxidase, glutathione reductase

(Ighodaro & Akinloye 2018).

Oxygen stress occurs gradually and causes persistent damage to individuals. Oxidative stress will lead to and aggravate many diseases, including hypertension, type 2 diabetes, atherosclerosis, and dementia **(Kitada, Ogura, & Koya, 2016; Buford, 2016; Chard et al., 2017).** Excessive redox-active free radicals can cause oxidative damage of biological macromolecules, leading to oxidative stress in the body, accompanied by the occurrence and development of random oxidative stress, increased production of hydrogen peroxide by mitochondria, and increased oxidative damage in the body **(Rao, 2009).** Redox regulation is an important issue in the study of oxidative stress. Maintaining redox balance and regulating redox-related genes are new strategies to alleviate oxidative stress **(Hohensinner et al., 2018).**

The oxidation model of D-galactose was established to verify the antioxidant effect of antioxidant active substances. It has been gradually applied to the research and development of antioxidant health products. Studies have shown that natural foods have strong antioxidant and free radical scavenging abilities due to their structural characteristics SOD is effective in preventing and treating diseases related to superoxide free radicals. When superoxide anion radicals are produced excessively or the SOD concentration is low, excessive superoxide anion will cause oxidation **(Lee, Hyun, Jenner, & Halliwell, 2001).** CAT is an antioxidant enzyme that mainly exists in erythrocytes and some tissue cells, as well as in mitochondria and the cytoplasm **(Selvaratnam & Robaire, 2016).**

In the process of normal oxidative respiration, organisms constantly produce ROS. As a highly active molecule, it contains unpaired electrons. The enzymatic system represented by SOD can remove ROS. SOD, as the first line of defense against ROS, mainly disproportionates O2 − to H2O2. CAT can decompose H2O2, produce H2O2, and increase the oxygen content in cells **(Sharma *et al*.,2012).**

Before and during 7 day of pregnant SOD show significant decrease ($p \leq 0.05$) in G2 group when compared to G1, G3 and G4. This result agreement with many

studies that used D-galactose to induced ROS in rat **(Tian *et al*.,2011; Peng *et al*.,2014; Haider *et al*.,2015)**.

SOD enzyme activity decreased in rat that received D-galactose**(Kalaz *et al*.,2014)** in normal condition increased this enzyme activity in normal rat **(Sun *et al*.,2007)** .

Increased SOD and CAT enzyme activities in normal state and decreased in D-galactose treated animals**(Ahangarpour *et al*.,2018)** . CAT is a primary antioxidant enzyme that converts H2O2 to water, so if CAT activity decreases in cells, it may lead to the accumulation of H2O2 and cause DNA damage or cell death **(Ho et al.,2001).** Thus D-galactose induced dysfunction through the enhancement of both SOD and CAT enzyme activity most likely as the result of a decline in free radicals (anion superoxide)**(Shan *et al*.,2009)**

other study showed that catalase and SOD can protect ß-cells against ROS-induced damage. Also, antioxidant treatment can suppress apoptosis of ß-cells and preserve ß-cell function in diabetic mice**(Fridlyand & Philipson 2006).**

The generation of ROS and oxidative stress are linked to pathophysiological changes throughout natural and D-gal-induced aging, and it is clear that rats of different ages react differently to D-gal therapy. The antioxidant enzyme activity and the amount of lipid peroxidation successfully depict and measure the pro-oxidant/antioxidant equilibrium. Changes in antioxidant enzyme activity in the D-gal model of accelerated aging are not only influenced by D-gal excess, but also rely on the developmental stage of the animals, as demonstrated by our research**.(Mehdi et al.,2021).** Hydrogen peroxide can also be reduced by catalase, which is present only in the peroxisome (Terlecky *et al* .,2006). In the mitochondria, GSH is particularly important because there is no catalase. Recent studies have shown that mitochondrial GSH is critical in defending against both physiologically and pathologically generated oxidative stress(Schieber & Chandel 2014). A selective reduction in the mitochondrial GSH pool has been reported in rats fed alcohol and may play an important pathogenetic role in the development of the liver disease **. (Sid *et al*.,2013).**

GSH is an important antioxidant in mammals that can scavenge the free radicals produced in cells, thereby reducing the damage of the cell membrane caused by the

formation of reactive oxygen species through lipid peroxidation (Berndt & Lillig, 2017). The interaction of various factors in the system can inhibit oxidative stress reaction of cells and slow down oxidation **(Vázquez-Medina *et al*., 2011).**

5.2.4 Effect of D-galactose and GSH on the Glucose and lipid profile in serum of female rat before and at 7 day of pregnant:

From our result before pregnant showed a significant increase in Glucose in D-galactose group this result agreement with **(Misra & Dey 2013;Badehnoosh et al.,2018; Dovom et al.,2019)**

There is considerable evidence that free radicals play an important role in the development of insulin resistance, impaired glucose tolerance, pancreatic islet cell dysfunction, and type 2 diabetes **(Allam & El Gazzar 2018).** Insulin secretion by isolated pancreatic islets revealed that the administration of D-galactose can induce ß-cell dysfunction and decrease secretion possibly by ROS generation**(Teodoro et al.,2021),**

Glucose is the most abundant nutrient crossing the placenta and the use of both glucose and amino acids by the fetus is essential to sustain intrauterine development **(Hay ,2006)** the major organs involved in the pathogenesis of metabolic dysfunction are pancreas and liver**(Fabbrini & Magkos 2015).** Metabolic syndrome is associated with aging, obesity, oxidative stress, insulin resistance, increased blood glucose, impaired glucose tolerance, increased insulin, decreased HDL, increased triglycerides, and increased LDL**(Bonomini *et al*.,2015).** Glucose is temporarily trapped inside the liver cells because phosphorylated glucose cannot diffuse back through the cell membrane **(Szablewski, 2011).**

our previous result at 7 day of pregnant rat showed significant increase in Glucose in the D-galactose group this result agreement with**(Nam *et al*.,2013; Sun *et al*.,2020;Hong *et al*.,2021)**.

D-galactose is a commonly used senescent agent in research that can be used to establish an oxidative stress animal model. A small amount of D-galactose can be converted into glucose and will participate in metabolism, but a large amount of D-galactose will lead to the disorder of cell metabolism, changes in the activity of oxidase in tissues and cells, and the production of many superoxide anions and oxidative products, resulting in oxidative damage to the structure and function of biological macromolecules and ultimately leading to oxidative stress **(Li et al., 2016).**

D-galactose is a reducing sugar, and when it accumulates in the body, it can react with the free amines of amino acids in proteins and peptides to form a Schiff base, an unstable compound. If this situation continues for subsequent months, the compounds are oxidized and become very stable. These are known as advanced glycation end products (AGEs) **(Hegab *et al*.,2012).** AGEs increase during ageing and have been regarded as one of the senescence markers, accumulating evidence proposes that AGEs are interacting with specific receptors (RAGE) in many cell types **(Frimat *et al*.,2017).**

Over-expressed RAGE activates NFkB and other inflammatory mediators that may be associated with metabolic disorders, aging and age-related diseases, especially affecting the pancreas and liver. A substantial amount of evidence has demonstrated that ROS and AGEs, produced after high concentration of D-galactose have been implicated in the pathological processes of age-related disease such as diabetes, arteriosclerosis **(Pickering *et al*.,2018; Nasoohi *et al*.,2018).** The present study was designed to explore the antioxidant and protective effects of GSH in rat with compromised pancreatic and hepatic function. The model selected was daily administration of D-galactose, a reducing sugar which can be converted into aldose and hydro peroxide this finding agreement with **(Potenza *et al*.,2021).**

Pregnancy is associated with significant changes in the functions of the normal liver and understanding these changes is essential to a proper clinical evaluation of liver abnormalities during pregnancy**(García-Romero *et al*.,2019).**

In the early pregnancy, glucose tolerance is normal or slightly improved and peripheral (muscle) sensitivity to insulin and hepatic basal glucose production is normal. Glucose production increases with maternal body weight, such that

glucose production per kilogram bodyweight does not change throughout pregnancy**(Khan *et al*.,2019).** A high increase in blood glucose during pregnancy could lead to gestational diabetes which is characterized by difficulty during delivery, abnormal fetal weight, adolescent obesity, and neonatal hypoglycemia **(Metzger *et al*.,2007).**

In our previous result of lipid profile parameters, in group who injected (IP) (100mg/kg) of D-galactose showed significant increase in total cholesterol, LDL-C, VLDL-C and TAG. And showed significant decrease in HDL-C this result agreement with many studies **(Yoo *et al*.,2012;Kumar & Rizvi 2014; Sahrir *et al*.,2017)** . The results of lipid profiles show that D-galactose did increase serum levels of triglyceride (TAG), low density lipoprotein (LDL-C), and very-low-density lipoprotein (VLDL-C),pregnancy hormones increased the serum total cholesterol level. Estrogen seems to be responsible for most of the alteration in lipoprotein metabolism during pregnancy. **(Phuse,2016).**

During the course of normal pregnancy, plasma triglyceride and cholesterol concentrations rise and as pregnancy progresses both become normal,hormonal variations during pregnancy affect lipid metabolism**(Ekhator & Ebomoyi 2012).** The endogenous female sex hormones have a significant effect on serum lipids.12 During pregnancy, there is an increase in the hepatic lipase activity and decrease in lipoprotein lipase activity,hepatic lipase is responsible for the increased synthesis of the TGs at the hepatic level, whereas the decreased activity of lipoprotein lipase is responsible for the decreased catabolism at the adipose tissue level . Also During early pregnancy, maternal metabolic environment is modified by a rise in serum levels of estrogen and progesterone, pancreatic beta-cell hyperplasia occurs and there is an increase in the secretion of insulin. Hyperinsulinemia leads to an increase in peripheral glucose utilization, a decline in fasting plasma glucose levels, increased tissue storage of glycogen, increased storage of fats and decreased lipolysis**(Plows *et al*.,2018;Casado *et al*.,2021)**

In early pregnancy there is an increased accumulation of fat depots in the mother, which is switched to an active adipose tissue breakdown in late pregnancy; these changes are responsible for the maternal hyperlipidemia that is normally present during the last third of pregnancy**(Herrera & Ortega-Senovilla 2014).** The changes are controlled by different hormones, but the biphasic changes in insulin

sensitivity taking place during pregnancy seem to play a major role. Maternal hyperlipidemia mainly results from an increase in TAG-rich lipoproteins **(Abdulateef & Mohammed 2019).**

lipid metabolism changes during pregnancy, natural rising of plasma lipids is seen in normal pregnancy, but this event is not atherogenic and it is believed this process is under hormonal control but in complicated pregnancy**(Reddy *et al*., 2018),** there is a possible defect in the mechanism of adjusting physiologic hyperlipidemia Plasma lipid profiles in the first trimester of pregnancy may predict the incidence and severity of pre-eclampsia **(Ayuba *et al*.,2018).** The anabolic phase of early pregnancy encourages lipogenesis and fat storage in preparation for rapid fetal growth in late pregnancy**(Grimes & Wild 2018)** Lipolysis is increased as a result of insulin resistance, leading to increased flux of fatty acids to the liver promoting the synthesis of very low-density lipoproteins (LDLs) and increased triglyceride (TG) concentrations **(Toescu *et al*.,2004).** Because of a decrease in the activity of lipoprotein lipase, very-LDL remains in the plasma for longer and leads to the accumulation of LDL. An increase in LDL is associated with the development of atherosclerosis, changes in lipid metabolism during gestation also play a key role in the development of fetal fat mass and subsequent growth **(Toescu *et al*.,2004)**

Cholesterol is an essential component of cell membranes, the precursor of bile acids and steroid hormones; it is required for cell proliferation, for the development of the growing body, for cell-to-cell communication and as precursor of regulatory agents. Consequently, embryonic and fetal cholesterol requirements are relatively high. During early gestation, maternal cholesterol actively contributes to fetal cholesterol, but during late pregnancy the main source appears to be fetal synthesis of cholesterol de novo **(Herrera & Ortega-Senovilla 2014; Zeng & Li 2017).** It is known that high concentrations of many of the steroids occur as normal pregnancy advances. Since cholesterol is the source of most of the steroids found in increased amounts in the circulation of normal pregnant patients, the part played by lipid metabolism in pregnancy**(Parchwani & Patel 2011).**Most authors believe that the increase in blood lipids is related to the requirements of the fetus and to development of the mammary apparatus **(Lenders *et al*.,2000;Asif *et al*.,2018)**

In pregnant woman, Lipid profile changes during pregnancy are a result of physiological adaptation to the state of pregnancy. There are increases in the blood concentration of Cholesterol, Triglycerides, LDL cholesterol, VLDL cholesterol and decreases in HDL cholesterol. [1],lipid profile is a group of tests that are used to determine risk of coronary heart diseases. **(Poveda *et al*.,2018;Adank *et al*.,2020)**

GSH decreasing serum levels of VLDL and TG. The corresponding results are in agreement with the present study showing that GSH can reduce harmful lipid factors and increase the serum level of HDL**(El-Demerdash & Nasr 2014)**.A high concentration of GSH protects cells against a variety of reactive oxygen species (dos Anjos *et al*., 2018) also many cell types and tissues have been reported to release GSH (Dringen & Hirrlinger2003). GSH seems to have essential functions in the extracellular space**.(Forman *et al*.,2009).**

5.3 Histopathological Changes

5.3.1Effect of D-galactose and GSH on the uterus of female rat at 7 day of pregnant: The results of the current study D-galactose used did not affect the implantation process during pregnancy, which is considered one of the basic processes in the success of pregnancy. Successful pregnancy requires coordination of three interrelated processes: the formation of the fetus and placenta and formation of the maternal tissue. One of the events observed in this study was the proliferation and differentiation of endometrial cells to form decidual cells in both the control rat group and the treatment groups, and it has been proven that early in pregnancy the decidual tissue that occurs in the endometrium is formed this leads to the acceptance by the uterus of the fetus. It is often stated that the formation of the decidual tissue is necessary for the protection and safety of the mother against trophoblast invasion **(Brosens *et al*.,2002).**

Stroma cells differentiate to become round and possess the characteristics of myofibroblasts **(Karahuseyinoglu *et al*.,2008).** One of the criteria and conditions that must be met for successful blastocyst implantation in rats is the appearance of the decidual tissue **(YoshInAgA,2012).**

The formation of the decidual tissue, which is one of the prominent signs of pregnancy, especially in animals with Haemochorial placenta, that any success of

the implantation process and the development of the fetus is directly related to the formation of the decidual tissue and a number of variables that occur in it **(Fonseca *et al*.,2012;Chavan *et al*.,2016).** Any comparison of the results obtained for the groups of rats D-galactose for the seventh days of pregnancy and comparing those results with the variables that occurred in the decidual tissue for the same period in the control group clearly shows the natural steps and stages in which the decidual tissue plays an important role in it and even in the formation and growth of the fetus in the subsequent periods and these It is seen in normal pregnancy in rats**(Liao *et al*.,2015; Li *et al*.,2017)** .

In fact, the presence of decidual tissue is one of the signs of embryo acceptance by the mother von **(Rango,2008).** . In this study, the sequence of events from successful implantation is reflected in the appearance of the decidual tissue on the seventh day of pregnancy in the antimesometrial side **(Fonseca *et al*.,2009).**

The appearance of the decidual tissue is part of the scheme that usually begins with the proper growth and formation of the endometrium, whether spatially or temporally, for implantation of the blastocyst **(Vinketova *et al*.,2016).** The hormonal balance between progesterone and estrogen increases the number of fibroblast cells in the endometrium, which are morphologically differentiated and functionally adapted to be ready for blastocyst implantation in rats and mice **(Ojosnegros *et al*.,2021),** the implantation of the blastocyst and in both types of animals above usually leads to a decidual reaction in the Antimesometrial side of the endometrium, where the blastocyst attaches to it at first **(Lee & DeMayo 2004).,** after the blastocyst attachment there will be invasion Trophoblast cells that stimulate the fibroblasts of the stromal cells in the Antimesometrial side to differentiate and become the primary decidual zone. As mentioned in the current study and previous studies, the primary decidual tissue region is characterized by the absence of blood vessels and close contact between the decidual cells by means of tight junctions, which were observed in previous studies with electron microscopy **(Mizugishi *et al*.,2007; Blois *et al*.,2011; Favaron *et al*.,2011).** Previous study showed that exposure to D-galactose in large quantities exceeding the normal limits of 300 mg/kg/day or adopting it in large quantities in the nutrition may reach 50% of the food. This leads to a decrease in the weight of the pregnant mother and may reduce the number of follicles in the ovaries of rats, and

increase In the occurrence of resorption of fetuses in the uterus, and may act to destroy the follicles in the stages before or after birth.

Glutathione shields eggs from damage caused by oxidative stress during folliculogenesis, and as such, egg quality are dependent on it. In fact, research has shown that oocytes with higher levels of intracellular glutathione produce healthier and stronger embryos**(Adeoye *et al*.,2018).**

Among the prominent results that were observed in the control group and the treated group was the presence of the decidual reaction, and it agreed with the study**(Bellofiore et al.,2017).** which indicated that the further expansion and successful formation of the fetus is closely related to changes in the decidual tissue, and by comparing the results of the seventh day of the Pregnancy It was clear that the endometrium has undergone marked changes that reflect a natural sequence of events that can be observed in a normal pregnancy in rats **(MHaddao et al.,2005).** The current study showed that the formation of decidual tissue from the endometrium is associated with radical changes that occur in the stroma cells. And the distribution of the Collagen fibers and the important event was that the stroma cells of the endometrium suffered modifications to turn into decidual tissue, which is an important indicator of the success of implantation in the anti-mesenteric part of the uterus. The cells of the decidual tissue are tightly packed, in addition to the presence of several connections between cells in this region, described as tight connections **(Aplin, 2008),** and Gap junctions **(Li *et al.*, 2017).** This is a reference to the barrier function of primary deciduous tissue for trophoblast invasion during the early stages of pregnancy after implantation **(Schumann *et al*.,2015).**

The disappearance of Collagen fibers on the seventh day in the primary decidual tissue, their reappearance in the secondary decidual tissue,This is in agreement with the findings of the researchers that a significant increase in the amount of Collagen fibers in the uterus of the pregnant mother occurs, up to 500%, compared to the uterus of the non-pregnant mother **(Burbank *et al*.,2009).**

The disappearance of microscopic fibers from some parts of the decidual tissues of rats in both the control and treatment groups raised the question that Matrix metalloproteinases (MMPs) enzymes may have a role in this, as **(Pijnenborg *et***

***al*.2011;Silvaa &Serakides 2016)** indicate the role of these enzymes secreted by migratory cytotrophoblasts, noted by **(Clark *et al.*2013)** Association of these enzymes (MMPs) with migratory cytotrophoblasts in this animal.

The results of the study revealed that the two drugs that were used did not affect implantation and formation of the decidual tissue, and that the rearrangement of the grafted fibers on the seventh day of pregnancy, which is necessary for the success of early pregnancy in all types of animals with hemochorial placentae **(Pijnenborg *et al.*, 2011).**

Providing adequate nutrition to the fetus is a key to the success of pregnancy, and the decidual tissue cells during pregnancy are of great importance in this matter, especially during its early stages. It comes as a result of the fact that these cells have several functions, including the production of nutrients for the fetus before the formation of its vascular system, which indicates that these cells contain glycogen to The nutrition of the fetus in the stage of implantation and before the formation of the uteroplacental blood circulation, which depends mainly on the content of glycogen in the decidual cells and secretions of the uterine glands. **(Wooding and Burton, 2008).**

The early accumulation of glycogen in the anti-mesometrium region was important to participate in providing nutrition to the fetus in its early stages after implantation in the endometrium, as it begins to gradually disappear with the expansion of the decidual tissue. This indicates its use in nutrition by the fetus, where glycogen is broken down and converted into glucose from Before and after the dilated decidual cells to be transferred to the implantation area by blood vessels in the decidual tissue of the mother, it has already been observed that the accumulation of glycogen resembles wings in rats **(El-Shershaby *et al.*, 1986)** and in Mink **(Dean *et al*., 2014).** It is supposed to be associated with the formation of the fetus in the future in this region.

The decidual tissue in the endometrium depends on glucose metabolism **(Frolova *et al*., 2011)** and glucose is the mainstay of energy in the muscle layer **(Rizzo *et al.*, 2011).** In rodents, the total concentration of glycogen in the uterus reaches a peak during the pre-estrus and estrus periods, and then decreases during implantation and early pregnancy **(Greenstreet and Fotherby, 1973).**

5.3.2 Effect of D-galactose and GSH on the ovary of female rat at 7 day of pregnant:

This study showed that there is a decrease in the average number of fetuses in the uterine horns on the seventh day of pregnancy compared with the control group, because d-galactose has the potential to negatively affect the migration of primary germ cells, and thus supplying the gonads with a small group of primary germ cells may constitute This is mainly due to the depletion or the lack of the number of follicles and thus the lack of mature eggs, which in turn affects the number of embryos that will implant in the lining of the uterus **(Meldrum *et al*.,2016)**

Whereas, the decrease in the average number of embryos belonging to rats that were injected with D-galactose and glutathione, because glutathione protects the eggs from damage resulting from oxidative stress during follicle formation and also depends The quality of egg production on it, and some research has shown that eggs containing higher levels of glutathione inside the cells produce healthier embryos than others. It can reduce oxidative stress by destroying free radicals harmful to the reproductive system**(Adeoye *et al*.,2018)** .In other studies, glutathione is shown to be an antiaging antioxidant that could have a possible impact on egg health, one of the cells most affected by the aging process **(Aversa *et al*.,2016)**

Glutathione shields eggs from damage caused by oxidative stress during folliculogenesis, and as such, egg quality is dependent on it **(Banerjee & Bhattacharya 2019).**

Classic galactosemia is caused by deficient activity of galactose-1-phosphate uridyl transferase (GALT). GALT is the second of the three enzymes in the Leloir pathway**.(Siva & Zayed 2020).**

Galactose is needed for energy metabolism and glycosylation of complex molecules. It may be derived from exogenous (dietary) sources, most importantly lactose from dairy products, or endogenous production**(Lai& Wierenga 2009).**Deficiency of the GALT enzyme leads to accumulation of galactose and its metabolites and results in secondary glycosylation abnormalities. The cause of POI in classic galactosemia is not yet understood**(Coelho *et al*.,2017).** Several mechanisms have been postulated, including direct toxicity of metabolites (i.e. galactose-1-phosphate), altered gene expression, or aberrant function of hormones and or receptors due to glycosylation abnormalities **(Fridovich-Keil *et al*.,2011).** It is also possible that not one, but several mechanisms act in unison to cause POI in classic galactosemia.**(Forges *et al*.,2006).**

In general, POI can be caused by either the formation of a smaller primordial follicle pool or more rapid loss of primordial follicles **(Nelson, 2009).** and there is evidence for both mechanisms in classic galactosemia. In classic galacto- semia there is some evidence that the follicle pool at birth is as large as in girls without this disease **(Coelho, 2014).**

Histological findings in two adults showed absence of ovarian paren- chyma and an extremely reduced number of folli- cles in one subject Consistent with the absence of estrogen production due to absent ovarian activity **(Gubbels *et al*.,2009;Gubbels *et al*.,2013)** , hypoplastic prepubertal uteri were observed in patients who did not receive estrogen supplementa- tion **(Nabhan *et al*.,2009).**

Animal studies suggest that galactose might already have a direct toxic effect in fetal life. After feeding preg- nant and lactating Sprague–Dawley rats a high galactose diet, the offspring had a significant and striking reduc- tion in the number of small follicles, **(Rubio-Gozalbo *et al*.,2010),**a reduced number of primordial germ cells (PGCs) and a conse- quently smaller gonadal size suggesting impaired germ cell migration, and a deficient complement of follicles in some of the exposed animals **(Wang *et al*.,2017).** Their results in isolated granulosa cells suggest that galactose exposure leads to an increased expression of p53, a protein

mediating intrinsic death pathways in cells **(Jiao *et al*.,2019).** A high galactose diet significantly decreased the number of healthy growing and antral follicles, whereas the number of primordial or total atretic follicles was not affected**(El Bakly *et al*.,2020)**,animal studies suggest that galactosemia results in increased follicle apoptosis with an accelerated follicle loss of either primordial follicles or maturing follicles as the cause of POI **(Liu *et al*.,2006)** .The ovarian dysfunction of galactosaemia may present transiently as a gonadotrophin-resistant ovary syndrome characterized by an alternation of periods with hypergonadotrophic failure and ovulatory cycles **(Hernández-Angeles& Castelo-Branco 2016).** Therefore, the chance of a spontaneous pregnancy cannot be completely ruled out during this initial phase of ovarian failure in galactosaemic patients**(Pedersen *et al*.,2018).**

The progressive nature of ovarian failure in galactosaemia is further illustrated by the description of a histologically normal ovary in a galactosaemic girl who died at the age of 5 days **(Sun *et al*.,2018),** The latter condition is characterized by the presence of follicles in the ovary that are unresponsive to gonadotrophins, and is usually referred to as `resistant ovary syndrome' (ROS) **(Seow *et al*.,2020).** The general consensus is that ROS is one pathway in the progression to irreversible ovarian failure regardless of the mechanism **(Parascandolo & Laukkanen 2019).**

Conclusion

From the results and discussion obtained from this study , it could be concluded that :

1-Intra peritoneal injection (IP)of 100mg/kg B.W. of D-galactose for 45 day caused oxidative stress and aging showed significant increase in the serum MDA and decrease in GSH,CAT,SOD activities before and during 7 day of pregnant.

2-Increase Glucose level between groups also LDL-C,VLDL-C,TAG and Cholesterol concentrations and significant depression in serum HDL-C before and during 7 day of pregnant

3- Showed changes in progesterone Hormone and estrogen concentration at 7 day of pregnancy also in FSH and LH before and during 7 day of pregnant.

4- The preventive role played by glutathione is important in the process of implantation of the blastocyst, and it helps to increase the number of embryos, especially when given in conjunction with the D-galactose, which decreased the number of embryos upon exposure to it.

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Recommendations

1-Further studies about protective role of GSH on the antioxidants of aging induced by D-galactose.

2-Immun histological of this study about aging induced by D-galactose on the germ cell in the female rats.

3-Electron microscopic study about aging in the parenchymal cell in ovaries.

4-Studing about correlation of biomarker with cardiac cell aging.

5-Further studies about aging effects on the all stages of pregnancy.

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Appendix I

1-Estimation of Follicle Stimulating Hormone (FSH) Concentration

REAGENT PREPARATION

1.Wash Buffer

Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at 2- 30°C for up to 60 days.

2. Working Substrate Solution – Stable for one year Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1 : Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C). Test Procedure should be performed by a skilled individual or trained professional

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of FSH-Enzyme Reagent solution to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 350µl of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

8. Add 0.100 ml (100µl) of working substrate solution to all wells

(see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). Always add reagents in the same order to minimize reaction time differences between wells

11.Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of follicle stimulating hormone in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate serum reference versus the corresponding FSH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Draw the best-fit curve through the plotted points.

4. To determine the concentration of FSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph,

find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.214) intersects the dose response curve at 43.2mIU/ml FSH concentration

Appendix II

3.4.2 Estimation of Hormone (LH) Concentration

REAGENT PREPARATION

1. Wash Buffer

Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.

2. Working Substrate Solution – Stable for one year Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

Test Procedure should be performed by a skilled individual or trained professional

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C

2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of LH-Enzyme Reagent to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

8. Add 0.100 ml (100µl) of working substrate solution to all wells

(see Reagent Preparation Section). Always add reagents in

the same order to minimize reaction time differences

between wells

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.

10.Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). Always add reagents in the same order to minimize reaction time differences between wells

11.Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of luteinizing hormone (LH) in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate serum reference versus the corresponding LH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Draw the best-fit curve through the plotted points.

4. To determine the concentration of LH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.005) intersects the dose response curve at 42.7 mIU/ml LH concentration

Appendix III

Estimation of Hormone Progesterone

Concentration

Working Enzyme Reagent - Stable for 1 year.

Measure 0.7 ml (700 µl) of '17-OH Progesterone Enzyme Reagent' and add to the vial containing Steroid Conjugate Buffer. Store at 2-8°C.

2. Wash Buffer Dilute contents of wash solution to 1000 ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note 1: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have

bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C). Test Procedure should be performed by a skilled individual or trained professional

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.025 ml (25 µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.050 ml (50 µl) of working 17α-OH Progesterone Enzyme Reagent to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix.

5. Add 0.050 ml (50 µl) of the 17α-OH Progesterone Biotin Reagent to all wells.

6. Swirl the microplate gently for 20-30 seconds to mix

7. Cover and incubate for 60minutes at room temperature.

8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

9. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

10.Add 0.100 ml (100 µl) of substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

11.Incubate at room temperature for twenty (20) minutes.

12.Add 0.050 ml (50 µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. The results should be read within fifteen (15) minutes of adding the stop solution. Note: Dilute the samples suspected of concentrations higher than 20ng/ml 1:1 and 1:5 with 17-OH Progesterone '0' ng/ml calibrator or male patient serum pools with a known low value for 17-OH Progesterone.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of

17-OH Progesterone in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate serum reference versus the corresponding 17-OH Progesterone concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Connect the points with a best-fit curve.

4. To determine the concentration of 17-OH Progesterone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.880) intersects the dose response curve at 1.41 ng/ml 17-OH Progesterone concentration (See Figure 1).

Appendix IV

Estimation of Hormone Estrogen

Concentration

8.0REAGENT PREPARATION

1. Wash Buffer Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note: Do not use reagents that are contaminated or have

bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C). Test Procedure should be performed by a skilled individual or trained professional

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.025 ml (25 µL) of the appropriate serum reference calibrator, control or specimen into the assigned well.

3. Add 0.050 ml (50µl) of the Estradiol Biotin Reagent to all wells.

4. Swirl the microplate gently for 20-30 seconds to mix.

5. Cover and incubate for 30 minutes at room temperature.

6. Add 0.050 ml (50µl) of Estradiol Enzyme Reagent to all wells.

Add directly on top the reagents dispensed in the wells.

7. Swirl the microplate gently for 20-30 seconds to mix.

8. Cover and incubate for 90 minutes at room temperature.

9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

10.Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

11.Add 0.100 ml (100µl) of substrate solution to all wells. Always add reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

12.Incubate at room temperature for twenty (20) minutes.

13.Add 0.050ml (50µl) of stop solution to each well and gently mix

for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

14.Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. The results should be read within fifteen (15) minutes of adding the stop solution.Note: Dilute the samples suspected of concentrations higher than 3000pg/ml 1:5 and 1:10 with estradiol '0' pg/ml calibrator or male patient serum pools with a known low value for estradiol.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of

estradiol in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate serum reference versus the corresponding estradiol concentration in pg/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Connect the points with a best-fit curve.

4. To determine the concentration of estradiol for an unknown,locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.202) intersects the dose response curve at (160pg/ml) estradiol concentration

Appendix V

Determination of Serum Malondialdehyde (MDA) concentration (µ mol /L):

Malondialdehyde was estimated by Thiobarbituric acid (TBA) assay method of Buege & Aust, 1978 on spectrophotometer .

Principle:

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of Thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm.

Stock TCA – TBA – HCl Reagent:

It was prepared by dissolving 15% W/V trichloroacetic acid and 0.375% W/V Thiobarbituric acid and 0.25N HCl to make 100 ml (2.1 ml of concentrated HCl in 100 ml). This solution was mildly heated to assist in the dissolution of TBA. Dissolved 15 gm TCA and 0.375 mg Thiobarbituric acid in 0.25 N HCl and volume was made up to 100 ml with 0.25 N HCl.

Procedure:

To 0.4 ml of serum, 0.6 ml TCA-TBA-HCl reagents were added. It was mixed well and kept in boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added to eliminate centrifugation. This absorbance of pink colour was measured at 535 nm against blank which contained distilled water in place of serum. In blank 0.4 ml distilled water and 0.6 ml TCA-TBA-HCl reagent was mixed and boiled. Blank was always taken.

Calculation:

extinction coefficient of MDA at 535 nm is = 1.56×105

MDA concentration = χ / 0.0624 nmol / ml

Appendix VI

Determination of serum Reduced Glutathione concentration

Reduced glutathione was measured following the method of Sedlak and Lindsay (1968). First, 3.0 mL precipitating solution containing metaphosphoric acid, Na2EDTA and NaCl was added to 2.0 mL of the sample. The mixture was centrifuged at 4500 × g for 10 min. 1.0 mL of supernatant was added to 4.0 mL of 0.3 M Na2HPO4 solution and 0.5 mM DTNB (5,5 -dithiobis-2-nitrobenzoic acid) was then added to this solution. Reduced glutathione was measured as the difference in the absorbance values of samples in the presence and the absence of DTNB at 412 nm. GSH value was calculated as nmol GSH/mg protein in the tissues and mmol GSH/g Hb in whole blood using the reduced glutathione as a reference (hemoglobin levels were estimated in whole blood using the Drabkins' solution)

Appendix VII

Determination of serum concentration of (cat)

Procedure

Catalase activity was assessed by incubating the enzymes ample in 1.0 ml substrate (65 mmol/ml hydrogen peroxide in 60 mmol/l sodium–potassium phosphatebuffer,pH7.4)at37 °C for three minutes. There action was stopped with ammonium molybdate. Absorbance of the yellow complex of molybdate and hydrogen peroxide is measured at374nm against the blank.

Reagents

1. Sodium, potassium phosphate buffer (50mM,pH7.4): this buffer isprepared by dissolving 1.1g of Na2HPO4 and 0.27g of KH2PO4 in 100ml distilled water.

2. H2O2 (20 mM) in 50mmol/l sodium, potassium phosphate buffer: this solution is freshly diluted and standardized daily using a molar extinction coefficient of 43.6M_1 cm_1 at 240nm.

3. Ammonium molybdate (32.4mmol/l).

3. Calculation The rate constant of a first-order reaction (k) equation is used to determine catalase activity:

t: time.

S°: absorbance of standard tube

S: absorbance of test tube.

M: absorbance of control test (correction factor).

Vt: total volume of reagents in test tube. Vs: volume of serum

Appendix VIII

Determination of serum concentration of (sod)

SOD activity determination

Preparation

1. Tris buffer (pH 8.0): was prepared by dissolving 0.258 gm of tris and 0.111 gm of Ethylenediaminetetraacetic acid (EDTA) in dH2O and completing the volume to 100 ml.

2. Pyragallol solution (0.2 mM): was prepared by dissolving 0.0252 gm of pyragallol with 10 ml of HCl and completing the volume to 100 ml with dH2O.

Procedure

According to Marklund and Marklund (1974), reaction mix is consisting of 50 µl crude enzyme extract with 2 ml of tris buffer and 0.5 ml of pyragallol (0.2 mM) which absorbs light at 420 nm. Control solution contains the same materials except for the enzyme extract that was replaced by dH2O. As a blank, dH2O was used. Single unit of enzyme is defined as the amount of enzyme that is capable of inhibiting 50% of pyragallol oxidation. SOD activity was calculated using the following equation (Ma et al., 2009) :

SOD activity (u/ml)= (Vp-Vs)/(Vp*0.5)*(Vt/Vs)*n

Vp=Auto oxidation rate of pyrogallol rate of pyrogallol (control)

Vs= Auto oxidation rate of sample (with enzyme)

Vt=Total reaction volume (ml)

Vs= volume of enzyme used for the assay (ml)

n= dilution fold of the SOD sample

0.5= factor for 50% inhibition

Appendix IX

Determination of Serum Total Cholesterol (TC) Concentration (mg/dl):

The procedure of evaluation of serum Total Cholesterol was described by Allain et al., 1974.

Procedure:

Contents	Blank	Standard	Sample
Cholesterol standard(s)	-----	10µl	-----
Sample	------	------	10µl
Reagent(A)	1.0mL	1.0mL	1.0mL

The mixture was thoroughly mixed and incubated for 5 minutes at 37˚ C. The absorbance of the sample (A sample) and the standard (A Standard) was measured against the blank' the absorbency color was read at 500 nm after 5 min at 37˚ C.

C) Calculation:

TC conc. (mg/dl) = A sample /A standard × 200

Appendix X

Triglyceride (TAG) concentration (mg/dl) .

The procedure of evaluation of serum triglycerides was described by (Fossati and Prencipe,1982).

Procedure:

Contents	blank (µl)	Standard (µl)	Sample (µl)
Triglycerides standard	---	10	---
Sample	---	---	10
Reagent(A)	1000	1000	1000

The mixture was carefully mixed and incubated for 5 minutes at 37 ºC. The absorbencies of the sample (A sample) and the standard (A standard) were measured against the blank. The absorbency color was read at 505 nm after 5 minutes at 37 ºC.

A) Calculation:

TG conc. (mg/dl) = A sample /A standard × 200

Appendix XI

Serum high-density lipoprotein- cholesterol concentration

(HDL) (mg/dl) .

The procedure was described by (Naito and Kaplan, 1984).

Procedure:

Pipette into labeled centrifuge tubes

Sample	0.2 mL
Reagent(A)	0.5 mL

Mix thoroughly and let stand for 10 minutes at room temperature. centrifuge at a minimum of 4000r.p.m. for 10 minutes. Carefully collect the supernatant. Pipette into labeled test tubes

Contents	blank (µl)	Standard (µl)	Sample(µl)

Distilled water	50	-	-
HDLCholesterol Standard(S)	-	50	-
Sample supernatant	-	-	50
Reagent(B)	1000	1000	1000

The mixture was incubated for 10 minutes at 37 ºC. The absorbencies of the sample (A sample) and the standard (A standard) were measured against the blank. The absorbency color was read at 500 nm.

C) Calculation:HDL-c conc. (mg/dl) = A sample /A standard ×52.5 ×1.7

Appendix XII

Serum low-density lipoprotein- cholesterol (LDL) concentration (mg/dL).

LDL-c conc. (mg/dL) =total cholesterol – Triglycerides conc. /5 + HDL

This formula is only valid when TG concentration not exceeds 400 mg/dl (Friedewald et al., 1972).

Appendix XIII

Serum very low-density lipoprotein-cholesterol VLDL concentration (mg/dl).

VLDL-c concentration was determined by dividing triglycerides values (mg /dl) on equation (Friedewald et al., 1972).

VLDL-c conc. (mg/dL) = Triglycerides conc. /5

Appendix XIV

Harris' Hematoxylin

General base colorant used to color the nucleus in a dark blue color its components are:

Subject Quantity

1	Hematoxylin powder	2.5 g
2	absolute ethyl alcohol	25 ml
3	Potassium alum AIK(SO4)2.12H2O or ammonia alum NH4AI(SO4)2.12H2O	50 gm
4	warm distilled water	500 ml
5	Red mercuric oxide	1.25 gm
6	Glacial acetic acid	20 ml

The stainer was prepared according to the following steps and based on (Suvarna et al., 2013):Dissolve hematoxylin with absolute alcohol, then add the dissolved alum to it in warm distilled water, put the mixture on fire until it boils, then add red mercuric oxide to it.

Appendix XV

Eosin stain

The stainer was prepared according to the following steps and based on (Suvarna et al., 2013)

	Subject	Quantity
1	eosin powder	1 gm
2	70% ethyl alcohol	99 ml
3	Glacial acetic acid	1 ml

Dissolve the eosin well in alcohol, then add glacial acetic acid to it and filter it before use the next day.

Tissue sections were stained using hematoxylin-eosin stained according to (Suvarna et al., 2013) as follows:

1- The tissue sections were placed in an oven at 60 °C for five minutes, then wax was removed from the slides using xylene, in two stages, for five minutes for each stage, then passed a descending series of ethyl

alcohol starting (100%, 100%, 90%, 80%, 70 %) for five minutes for each concentration

2- Tissue sections were placed in Harris hematoxylin stain for five minutes.

3- Washed with running water for ten minutes.

4- Paint with eosin for seven minutes.

5- Washed with distilled water for two minutes.

6- Then it was transferred to an ascending series of ethyl alcohol (70%, 80%, 90%, 100%, 100%) for two minutes in each concentration except for the last concentration and was placed in it for five minutes and then quenched with xylene in two stages in each stage for three minutes. .

Appendix XVI

Gomori's One-Stap Trichrome Stain

This stain was prepared according to Hansen (2006) method, where it is used to color the colloidal fibers and smooth muscle fibers, as the collagen fibers appear in green color, while the smooth muscle fibers, the nucleus and the cytoplasm are colored red, the colorant consists of the following components.

Subject	Quantity
1	Chromotrope 2R 0.6 gm
2	Light Green 0.3 gm
3	Glacial Acetic Acid 1 ml
4	Phosphotungstic Acid 0.8 gm
5	Distilled Water 100 ml

The components of the dye were dissolved in distilled water in order, then concentrated hydrochloric acid HCl 1 ml was added to it. It was placed in the refrigerator for 24 hours at 4 °C before use and filtered before use with filter paper.

Coloring method:

1- Remove the wax from the sections and pass a series of ethyl alcohols down to the distilled water, as in the previous staining steps.

2- The tissue sections were placed in Bouin's solution for one hour in an oven at 56 °C and left to cool.

3- I washed it with running water until the yellow color disappeared from it.

4- Tissue sections were stained with hematoxylin for two minutes as a counter stain.

5- Washed with running water for five minutes.

6- Tissue sections were colored with Trichrome for 15-20 minutes.

7- It was placed in acetic acid at a concentration of 0.2% for half a minute.

8- Blot work means placing each section between two filter papers and gently wiping them with the palm of the hand.

9- The tissue sections were washed with distilled water and then passed with ethyl alcohol at a concentration of 90% for one time and 100% for two changes for two minutes.

10- I lathered with xylene twice for five minutes.

الخلاصة

أجريت هذه الدراسة للتقصي عن الدور الوقائي للكلوتاثيون على مبيض اناث الجرذان البيض المعرضة لل D-galactose /جامعة الصرفة للعلوم التربية وكلية الطب البيطري كلية في مختبرات أجريت الدراسة . كربلاء في المدة من تشرين الأول 2020 لغاية حزيران 2021 ، استخدمت حيوانات الجرذان البيض ( ) بـ IPوعددها 30 جرذاً، منها عشرة ذكور للتلقيح فقط أما الإناث وعددها 20 جرذاً تم حقنها داخل الصفاق (لمدة 45 يومًا.GSH و D-galactose100 ملغم / كغم من وزن الجسم

5 / مجموعة) ، تم حقن المجموعة الأولى (تم تقسيم عشرين (20) من اناث الجرذ إلى أربع مجموعات) بالمحلول الملحي الفسيولوجي0.9% داخل الصفاق لمدة ستة أسابيع وعدت كمجموعة تحكم ، وتم حقن G1(من وزن الجسم . D-galactose ملغم / كغم 100) (IP) داخل الصفاق G2جرذان المجموعة الثانية (ملغم / كغم كلوتاثيون من وزن الجسم ، تم 100) داخل الصفاق G3بينما تم حقن حيوانات المجموعة الثالثة ( ) بنسبة 100 ملغم / كغم كل من الكلاكتوز IP) داخل الصفاق (G4حقن حيوانات المجموعة الرابعة (والكلوتاثيون من وزن الجسم.

تم جمع عينات الدم بعد منع الطعام عن الحيوانات لمدة ثمانية ساعات بتقنية طعنة القلب بعد45 يوم من التجربة لقياس: المؤشرات الحيوية للإجهاد التأكسدي بما في ذلك تركيز الكلوتاثيون في الدم والمانولديهايد والسوبراوكسايد دايستيز والكاتاليز, كذلك قياس تركيز هرمون المحفز لنموالجريبات ، والهرمون اللوتيني ، وهرمون الاستروجين والبروجسترون ومعرفة صورة الدهون في الدم ، البروتين الدهني منخفض الكثافة) ، الدهون الثلاثية ، الكوليسترول والبروتين الدهني HDL) ، البروتين الدهني عالي الكثافة (LDL( ) ، وتقدير تركيز الكلوكوز بالدم قبل وبعد الحمل ودراسة التغيرات النسيجية VLDLمنخفض الكثافة جدًا (لأنسجة الرحم والمبيض في اليوم السابع من الحمل.

مقارنة مع G2 في مستوى الجلوكوز في ($p\leq0.05$) أظهرت نتائج الدراسة قبل الحمل زيادة معنوية في VLDL-C و LDL-C و TAC و TC و MDA كما أظهرت زيادة معنوية في G4 و G3 و G1 مقارنة مع المجموعات الأخرى ، كما تبين ان استروجين مصل الدم حصل فيه زيادة معنوية G2 مجموعة و SOD.CAT و HDL-C مقارنة مع مجموعة التحكم. وأظهر انخفاضًا معنويًا في G2 في ($p\leq0.05$) انخفاضًا معنويًا FSH ، أظهرت نتائجG4 و G3 و G1 مقارنة بالمجموعات G2 في GSH انخفاضًا معنويًا LH ، بينما أظهرت نتائجG3 و G1 مقارنة بالمجموعات G2 في مجموعة($p\leq0.05$) مقارنة بالمجموعات الأخرى ، وكذلك البروجسترون في الدم اظهر انخفاضًا G4 و G3 في ($p\leq0.01$) G4. و G3 و G1 عند مقارنتها بالمجموعات G2 في مجموعة ($p\leq0.05$) معنويًا

LH في مصل ($p\leq0.05$) بينما أظهرت النتائج في دراستنا بعد سبعة أيام من الحمل انخفاضًا معنويًا ، أيضًا انخفاض معنوي في هرمون G4 و G3وG1 مقارنة بالمجموعات G2 في مجموعة FSH ومستوى مقارنة مع المجموعات الأخرى، أظهر البحث G2 الاستروجين والبروجستيرون في الدم في المجموعة مقارنة G2 في المجموعة CAT و SOD و GSH تركيز HDL-C انخفاضًا ملحوظًا في مستوى

و TC في مستوى مصل البروجسترون و ($p\leq0.05$) بالمجموعات الأخرى ، بينما أظهر زيادة معنوية G4. و G3 و G1 مع مجموعات G2 في تجميع مجموعة MDA و VLDL-C و LDL-C و TAG

على المبيض ، حيث أثر على معدل التبويض وعدد D-galactose أما التغير النسيجي فكان بتأثير البصيلات الناضجة في المبيض، ومن ناحية أخرى لم يكن له تأثير على انغراس الأجنة في رحم الجرذان الحامل ، ولاحظنا في المقاطع النسيجية للمبايض والرحم للمجموعات التي عولجت بالجلوتاثيون ، كانت فقط ، كان المبيض في ذروة نشاطه ، وكان الرحم والانغراس في D-galactose النتائج على النقيض من الشكل الصحيح.

الزائد في المبيض قبل D-galactose ضد GSHفي الختام ، تؤكد نتائج هذه الدراسة الدور الوقائي لـ وأثناء الحمل.

جمهوريه العراقية
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء /كلية الطب البيطري
فرع الفسلجة والكيمياء الحياتية والادوية

دراسة فسيولوجية نسيجية مرضية لتأثير الكلوتاثايون قبل وخلال الحمل المبكر للجرذان المعرضة الى دي كلاكتوز

رسالة مقدمة الى

مجلس كلية الطب البيطري جامعة كربلاء وهي جزء من متطلبات نيل-

درجة الماجستير في علوم الطب البيطري / الفسلجة والادوية والكيمياء الحياتية

من قبل

زينة غسان محمد صالح مهدي

بكالوريوس طب وجراحة بيطرية/ كلية الطب البيطري/ جامعه كربلاء

2016-2015

بإشراف

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2021م 1443 هـ