DEVELOPMENTAL TOXICITY EFFECTS OF
ATORVASTATIN AND ROSUVASTATIN IN MICE

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DECLARATION

I, David Musorowegomo, certify that this dissertation is my original work and has been
prepared in accordance with guidelines for the Masters of Clinical Pharmacology Program,
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ABSTRACT

Background: Statins reduce the risk, morbidity and mortality associated with cardiovascular
events, and generally considered safe to use but their safety in pregnancy is not known.
Statins are considered potentially teratogenic and are contraindicated in pregnancy on the
basis of lack of evidence.

Objectives: The objectives of the study were to determine and compare the developmental
toxicity effects including teratogenic and offspring weight effect of rosuvastatin and
atorvastatin in time mated mice.
Methods: Fifty six Balb c mice were divided into seven experimental groups of eight mice each. Atorvastatin and rosuvastatin were administered at doses of 10, 40, 100mg/kg/day via oral gavage route once every day for 7 days prior to the mating and continued during the mating and pregnancy period up to delivery. Maternal weight changes and miscarriages were monitored during the pregnancy period. The mice were allowed a vaginal delivery and the offspring were weighed and assessed for gross morphological defects.

Results: There was no dose related changes in litter size or suppressed maternal weight gain in the study groups. No significant differences when the control group was compared to the individual atorvastatin groups, rosuvastatin 10 and 100mg/kg \( p > 0.05 \). The rosuvastatin 40mg/kg group had significantly lower birth weight compared with control \( p = 0.022 \) in post hoc analysis. No gross morphological defects were observed in all the offspring.

Conclusions: No developmental toxicity including teratogenic effects were observed on atorvastatin and rosuvastatin at 10, 40 and 100mg/kg in a mice model.

Key search words: Developmental toxicity, teratogenicity, statins, atorvastatin, rosuvastatin,
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CHAPTER 1: INTRODUCTION

1.1 Problem statement

Statins are commonly used as lipid lowering agents. Since their approval in 1987, statins have shown ability to reduce the risks of vascular death, non-fatal myocardial infarction, stroke, and the need for arterial revascularisation procedures in several large, high-quality randomised trials (1). Statins are effective cholesterol-lowering drugs that reduce the risk of cardiovascular disease events (2)(3). The beneficial effects of statins may extend beyond their effects on serum cholesterol levels. Some of the cholesterol-independent or “pleiotropic”
effects of statins involve improving or restoring endothelial function, enhancing the stability of atherosclerotic plaques, and decreasing oxidative stress and vascular inflammation(4). Statins enjoy widespread acceptance as effective drugs in reducing morbidity and mortality in patients with or without cardiovascular disease and are considered safe for long term use. However, their safety in pregnancy is not known and are generally considered potential teratogens on the basis of lack of evidence on their safety(5). Human teratogenic risk has not been proven nor has it been ruled out by the available data on statin use in pregnancy. Possible differences in risk between individual statins require further evaluation(6).

There are no evidence-based guidelines with respect to the use of statins in pregnancy. Further, no observational or interventional studies have been conducted in humans to evaluate the effect of statins during pregnancy. Most information considered is experience-based instead of evidence-based. On the basis of the limited animal data, the Food Drug Administration (FDA) in the United States assigned statins to pregnancy category X (which states that risk involved in use of the drug in pregnant women clearly outweigh potential benefits). The FDA cited lack of study data relating to the effects on a pregnant women and/or the foetus (as opposed to the existence of evidence of harm) as the main reason for this decision(7).

With the growing number of women of child bearing age with hypercholesteremia who require a statin during pregnancy, safety data on prenatal exposure to statins is urgently needed(8). If people get pregnant whilst on statins, there are no clear cut guidelines on what to do with the pregnancy. This causes a great deal of dilemma to a pregnant mother who requires a statin. If the statins were to be discontinued during pregnancy this may be harmful to both mother and the child. International guidelines emphasize the need to achieve
recommended low density lipoprotein cholesterol (LDL-C) in order to reduce morbidity and mortality associated with coronary heart disease(8).

Since initial pre-marketing studies of lovastatin in animals, teratogenesis has been assumed to be a class wide function of statins' mechanism of action(6). Although the current trend is that actual risk is lower than once thought, the available literature is limited by potential reporting bias and frequently lacked numbers of total exposures to statins during pregnancy with reported malformations(6). Further, no human studies have evaluated the two newest statins (i.e. rosuvastatin and pitavastatin). Conversely, most studies that have implicated statins as potential teratogens have been on older lipophilic statins (i.e. lovastatin and simvastatin)(6).

Very few clinical and laboratory data are available regarding inadvertent exposure to statins in human pregnancy(9)(10). The FDA identified 178 spontaneous reports of exposure to statins during pregnancy between 1987 and 2001, including 52 cases of exposure in the first trimester (11). Among these cases, there were 20 reports of malformations, including four severe defects involving the central nervous system and five unilateral limb deficiencies. The two simvastatin-exposed cases of limb deficiency described complex lower-limb anomalies, including both long-bone shortening and aplasia or hypoplasia of the foot structures. The infant in one of these cases and a lovastatin-exposed infant also had rare forms of the vertebral-anal-cardiac-tracheal-oesophageal-renal-limb association(11).

A report based on the Merck pharmacovigilance data base of exposure to simvastatin or lovastatin during pregnancy identified 477 reports of exposure (12). Of these reports there were 225 documented outcomes: 154 were live born infants, 49 were elective abortions, 18 were spontaneous abortions, and four were foetal deaths. Six congenital anomalies were reported; one chromosomal translocation, one trisomy 18, one hypospadias, one duodenal atresia, one cleft lip, and one skin tag. The rate of congenital anomalies was 3.8%, which is
similar to the 3% background population rate. No specific patterns of anomalies were identified. Although the number of reports was relatively small, there was no evidence of an increase in congenital anomalies in children born to women exposed to simvastatin or lovastatin compared with the general population (12). Conclusions based on data obtained from spontaneous case reports have to be drawn with care, given the significant reporting bias in spontaneous retrospective reporting processes. Pregnancies that result in the birth of a malformed child are several times more likely to be reported than pregnancies with a healthy outcome (11).

Animal studies have produced conflicting evidence on the potential teratogenicity of older statins. Studies in rats and rabbits failed to show a teratogenic effect of simvastatin (11). However, skeletal malformations were observed with other structurally related HMG-CoA reductase inhibitors (lovastatin and active metabolites, cerivastatin, fluvastatin) in similar animal models (11). In addition, reports in which an excess of congenital anomalies were reported in statin treated rodents used excessive doses compared with the doses commonly prescribed in human subjects (5). Furthermore, no study has assessed the effect of different doses of statins within the therapeutic range on the birth weight of offspring. Theoretical concerns over a potential impairment in gonadal steroidogenesis by the decrease of cholesterol availability have never been supported by conclusive evidence in animals (11). No animal studies have been done on developmental toxicity of the newer statins like rosuvastatin and pitavastatin.

Studies in rats and rabbits have provided evidence that atorvastatin has developmental toxicity, but only at doses that induced maternal toxicity (13). Rats and rabbits (given 300 mg/day and 100 mg/day of atorvastatin, respectively) had smaller pups, reduced litter size, reduced maternal body weight and food consumption. No malformations were observed (13). Evidence of foetal anomalies (predominantly skeletal defects: vertebrae, sternum, fused ribs,
incomplete ossification) was found in rats treated with a HMG-CoA reductase inhibitor, mevinolin, at the high dose of 800 mg/kg/day(14). At these high doses, mevinolin also produced significant maternal toxicity. The relevance of the mevinolin-induced malformations in the context of treatment with other statins in clinical use is not clear(14).

1.2 Study significance

One of the relatively newer statin, rosuvastatin, offers the greatest lipid lowering efficacy at the lowest dose of treatment(15). Rosuvastatin has a favourable pharmacological profile including its selective uptake by hepatic cells, hydrophilic nature of metabolism by CYP3A4 isoenzymes which means adverse effects are low in those requiring concomitant therapy of statins with agents metabolised by CYP3A4 (16). Rosuvastatin typically inhibits HMG-CoA reductase and cholesterol synthesis to a significantly greater extent than other statins(17), but
it is not known whether this property makes rosuvastatin a more potential teratogen than other statins since no animal studies have been done to ascertain the teratogenic potential difference of this drug to the rest of the statin class of drugs.

Atorvastatin is one of the most commonly prescribed drug for dyslipidemia the world over (18). Lipophilic statins like atorvastatin equilibrate between maternal and embryonic compartments making them more teratogenic potentials than hydrophilic statins. A relatively higher dose of atorvastatin is usually required to achieve the LDL therapeutic goals in hyperlipidemia as compared to rosuvastatin(19), but it is not known if the differences in the dose levels of atorvastatin within its therapeutic range increases the teratogenic potential of the drug.

1.3 Purpose Statement

The purpose of the study was to determine the developmental toxicity of different therapeutic doses of atorvastatin and rosuvastatin in a pregnant mouse model. The study also assessed the changes in foetal weight and examined offsprings for gross morphological abnormalities.

CHAPTER 2: LITERATURE REVIEW

2.1 Epidemiology of Coronary Heart Disease and Dyslipidemia

Cardiovascular disease (CVD) today is responsible for approximately one-third of deaths worldwide(20). Developing countries account for the greater number of these deaths which is approximately 80% of global cardiovascular death(21). Coronary heart disease (CHD) is the greatest single cause of mortality and loss of disability-adjusted life years worldwide and a substantial portion of this burden falls on low- and middle-income countries (LMICs).
Deaths from CHD and acute coronary syndrome (ACS) occur on average at younger ages, in LMICs than in high-income countries, often at economically productive ages and frequently affect the poor within LMICs(22). One in every six adults (16.3% of the U.S. adult population) has high total cholesterol levels, and are at double the risk of heart disease compared with people with optimal levels(23).

2.2 Risk factors

The risk factors that are causally linked to cardiovascular diseases and coronary heart disease include smoking, obesity, hypertension, elevated LDL, low HDL, diabetes, physical inactivity. Some of the risk markers that show association with coronary heart disease but with no direct causal relationship include low socio economic status, elevated prothrombotic factors, markers of infection or inflammation, elevated lipoprotein (a) and elevated homocysteine(24).

2.3 Pathogenesis

Exposure to risk factors such as hyperlipidemia, smoking, hypertension and diabetes predisposes arterial atherosclerosis. Atherosclerosis, the main cause of CHD, is an inflammatory disease in which immune mechanisms interact with metabolic risk factors to initiate, propagate, and activate lesions in the arterial tree(25). Atherosclerosis results in endothelial injury and formation of atherosclerotic lesions (atheroma) (25).

A partial or complete occlusion of the coronary artery by ruptured atheromatous plaque associated with platelet activation and thrombosis results in myocardial ischemia which is the
hallmark or coronary artery disease. While most plaques remain asymptomatic with subclinical disease some become obstructive resulting in stable angina and a few become thrombosis prone and may lead to ACS(26).

Myocardial infarction occurs when the atheromatous process prevents blood flow through the coronary artery. Activation of plaque rather than stenosis precipitates ischemia and infarction. Coronary spasm may be involved to some extent, but most cases of infarction are due to the formation of an occluding thrombus on the surface of the plaque, rupture of the plaque and endothelial erosion being the major determinants of thrombus formation(25).

Coronary heart disease is nearly always caused by coronary atherosclerosis with or without luminal thrombosis and vasospasm. Atherosclerosis alone may cause stable angina but is rarely fatal. In contrast, thrombosis plays a major role in the pathogenesis of the life-threatening acute coronary syndromes (ACS), including ST-segment elevation myocardial infarction (STEMI), non-STEMI, and unstable angina(26).

2.4 Guidelines for managing hyperlipidemia

European and US guidelines now recommend lower LDL-C levels, particularly in high-risk patients(27) (28). Lipid-lowering agents have been shown to reduce morbidity and mortality associated with coronary artery disease (CAD) in all patients (29). However, these agents are more cost-effective in high-risk patients(29). Furthermore, there is greater risk reduction in those subjects achieving lower low-density lipoprotein cholesterol (LDL-C) levels (i.e., lower is better). The identification and aggressive treatment of these patients should therefore be a high priority for clinicians. Guidelines from medical organizations, such as the Adult Treatment Panel (ATP) III of the US National Cholesterol Education Program (NCEP),
emphasize that patients with CAD, diabetes, or global risk of CAD >20% over 10 years and LDL-C levels >130 mg/dL should receive drug therapy with a goal of reducing LDL-C levels to <100 mg/dL. The United Kingdom's Heart Protection Study (HPS) strongly suggested that even those with CAD or who are at high risk and LDL-C levels >100 mg/dL would benefit from drug therapy\textsuperscript{(29)(30)}.

ATP III treatment algorithm states that, in high-risk persons, the recommended LDL-C goal is <100 mg/dL, but when risk is very high, an LDL-C goal of <70 mg/dL is a therapeutic option, this therapeutic option extends also to patients at very high risk who have a baseline LDL-C < 100 mg/dL. Moreover, when a high-risk patient has high triglycerides or low high-density lipoprotein cholesterol (HDL-C), consideration can be given to combining a fibrate or nicotinic acid with an LDL-lowering drug. For moderately high-risk persons (2+ risk factors and 10-year risk 10% to 20%), the recommended LDL-C goal is <130 mg/dL, but an LDL-C goal <100 mg/dL is a therapeutic option on the basis of trial evidence. The latter option extends also to moderately high-risk persons with a baseline LDL-C of 100 to 129 mg/dL. When LDL-lowering drug therapy is employed in high-risk or moderately high-risk persons, it is advised that intensity of therapy be sufficient to achieve at least a 30% to 40% reduction in LDL-C levels. Moreover, any person at high risk or moderately high risk who has lifestyle-related risk factors (e.g. obesity, physical inactivity, elevated triglycerides, low HDL-C, or metabolic syndrome) is a candidate for therapeutic lifestyle changes to modify these risk factors regardless of LDL-C level\textsuperscript{(31)(32)}.

The American Heart Association and the American College of Cardiology (AHA-ACC), in their guidelines in November 2013 state that Low-density lipoprotein targets for treatment have been removed. The 10-year cardiovascular disease (CVD) risk is used as a reference treatment threshold of LDL levels between 2.0 and 5.0 mmol/L. Low-density lipoprotein levels are referenced only as extremes of the primary prevention spectrum and are no longer
used as thresholds for intervention. If drug treatment is indicated, the decision becomes whether to use a moderate- or high-dose statin. Lipid levels are part of global CVD risk assessment, but are otherwise not relevant to treatment type or intensity. Risk reduction using drugs involves statin therapy. No other drugs added to statins are believed to improve hard CVD end points. High-sensitivity C-reactive protein levels are not part of the treatment decision(33).

2.5 Drugs used in hyperlipidemia

2.5.1 Statins

The conversion of 3-hydroxyl -3-methylglutaryl Co-enzyme A to mevalonic acid is the rate limiting step in cholesterol synthesis. The mechanism of action of statins involves inhibition of the enzyme which catalyses this reaction, 3HMG-CoA reductase hence, interrupting the cholesterol synthesis pathway in the liver(34). Statins also increase the expression of Low Density Lipoprotein (LDL) receptors in hepatocytes leading to a reduction in circulating LDL cholesterol(35). Statins also tend to reduce the production of apolipoprotein B, leading to
reduced VLDL (very low density lipoprotein) secretion from the liver(36). The reduction of
plasma total cholesterol and LDL cholesterol levels induced by these statins result in
significant reduction in cardiovascular risk by reducing atherosclerosis in all major arterial
trees. Lowering of LDL-cholesterol by 25 to 30% with statins resulted in a highly significant
reduction of coronary event rates(37).

Six statins are available in most parts of the world: lovastatin (1987), simvastatin (1988),
pravastatin (1991), fluvastatin (1994), atorvastatin (1997), rosuvastatin (2003), and
pitavastatin (2003) (38)(39). Cervastatin was approved in 1998 but then withdrawn in 2001
because of a high risk of rhabdomyolysis(40).

2.5.2 Pleiotropic effects of statins

The overall clinical benefits observed with statin therapy appear to be greater than what
might be expected from changes in lipid profile alone, the beneficial effects of statins may
extend beyond their effects on serum cholesterol levels (41). Experimental and clinical
evidence indicate that some of the cholesterol-independent or “pleiotropic” effects of statins
involve improving or restoring endothelial function, enhancing the stability of atherosclerotic
plaques, and decreasing oxidative stress and vascular inflammation(41). Many of these
pleiotropic effects of statins are mediated by their ability to block the synthesis of important
isoprenoid intermediates, which serve as lipid attachments for a variety of intracellular
signalling molecules, in particular inhibition of small GTP-binding proteins, Rho, Ras, and
Rac, whose proper membrane localization and function are dependent on isoprenylation, may
play an important role in mediating the pleiotropic effects of statins(41).

2.5.3 Fibrates

Fibrates, a widely used class of lipid-modifying agents, results in a substantial decrease in
plasma triglycerides and is usually associated with a moderate decrease in LDL cholesterol
and an increase in HDL cholesterol concentrations (42). The effects of fibrates are mediated, at least in part, through alterations in transcription of genes encoding for proteins that control lipoprotein metabolism (42).

Fibrates activate specific transcription factors belonging to the nuclear hormone receptor superfamily, termed peroxisome proliferator-activated receptors (PPARs). The PPAR-α form mediates fibrate action on HDL cholesterol levels via transcriptional induction of synthesis of the major HDL apolipoproteins, apoA-I and apoA-II. Fibrates lower hepatic apoC-III production and increase lipoprotein lipase—mediated lipolysis via PPAR. Fibrates stimulate cellular fatty acid uptake, conversion to acyl-CoA derivatives, and catabolism by the β-oxidation pathways, which combined with a reduction in fatty acid and triglyceride synthesis, results in a decrease in VLDL production (42).

In general, fibrates are considered to be well tolerated, with an excellent safety profile. Common side effects include gastrointestinal tract disturbances, elevated liver enzymes and a rash, rhabdomyolysis is a rare side effect which occurs mainly in renal failure patients (43).

2.5.4 Bile acid binding resins

Reduce cholesterol by interrupting the enterohepatic circulation stimulating a concentration gradient resulting in the increased synthesis of bile acid from cholesterol (44). Increased conversion of cholesterol to synthesize bile acids results in a compensatory up regulation in hepatic LDL receptors, increased hepatic LDL-C uptake, and decreased circulating LDL-C (45). Resins reduce the endogenous bile acid pool by approximately 40%, which lowers LDL-C by 15 to 26% (44). Resins’ common side effects are flatulence constipation and
dyspepsia. Three types of resins are currently in clinical use cholestyramine, colestipol and colesevelam (44).

2.5.5 Niacin

Niacin favourably affects apolipoprotein B containing lipoproteins (VLDL and LDL) and increases apo A-I containing lipoproteins HDL. Niacin directly and noncompetitively inhibits hepatocyte diacylglycerol acyltransferase-2, a key enzyme for triglycerides synthesis. The inhibition of triglycerides synthesis by niacin results in accelerated intracellular hepatic apo B degradation and the decreased secretion of VLDL and LDL particles(46). Flushing is the most commonly reported side effect with niacin other side effects being elevated liver enzymes, hyperuricemia, nausea and hepatitis.

2.5.6 Ezetimibe

Ezetimibe is a cholesterol lowering agent which acts at the level of the brush border of the small intestines in the gastrointestinal tract. Ezetimibe blocks the absorption of dietary and biliary cholesterol and plant sterols resulting in intracellular cholesterol depletion by targeting the Niemann-Pick C1-like transporter resulting in a decrease in LDL(47). It is commonly used in combination with statins is well tolerated, and some of its common side effects are gastrointestinal related (47).

2.6 FDA Rating System for Teratogenicity

2.6.1 Category A

Studies in pregnant women have not shown that the drug increases the risk of foetal abnormalities if administered during the first second, third, or all trimester(s) of pregnancy. If this drug is used during pregnancy, the possibility of foetal harm appears remote. Because
studies cannot rule out the possibility of harm, however, the drug should be used during pregnancy only if clearly needed(48).

2.6.2 Category B

Animal reproduction studies have failed to demonstrate a risk to the foetus and there are no adequate and well-controlled studies in pregnant women. "Reproduction studies have been performed in animal at doses up to [X] times the human dose and have revealed no evidence of impaired fertility or harm to the foetus due to the drug. There are however no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed (48).

2.6.3 Category C

Animal reproduction studies have shown an adverse effect on the foetus, there are no adequate and well-controlled studies in humans, and the benefits from the use of the drug in pregnant women may be acceptable despite its potential risks. "The drug has been shown to be teratogenic (or to have an embryocidal effect or other adverse effect) in species when given in doses [X] times the human dose. There are no adequate and well-controlled studies in pregnant women. The drug should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus(48).

2.6.4 Category D

Positive evidence of human foetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but the potential benefits from the use of the drug in pregnant women may be acceptable despite its potential risks (for example, if the drug is needed in a life-threatening situation or serious disease for which safer drugs cannot be used or are ineffective). Under the warnings and precautions section, the drug can cause foetal
harm when administered to a pregnant woman. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be appraised of the potential hazard to a foetus(48).

2.6.5 Category X

Studies in animals or humans have demonstrated foetal abnormalities or there is positive evidence of foetal risk based on adverse reaction reports from investigational or marketing experience, or both, and the risk of the use of the drug in a pregnant woman clearly outweighs any possible benefit (for example, safer drugs or other forms of therapy are available). If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be appraised of the potential hazard to a fetus(48).

2.7 Statins and Teratogenicity

2.7.1 Hydroxymethylglutaryl-coenzyme A reductase inhibition mechanisms of teratogenesis

The mevalonate pathway is a complex pathway with cholesterol as an essential product. In embryonic tissues, cholesterol is needed for normal growth patterns, signalling domains in
plasma membranes, synthesis of steroid hormones and activation of Hedgehog morphogen(49). Since Hedgehog proteins act as key regulators of embryonic growth, patterning and morphogenesis of many structures, down-regulation of the synthesis of these proteins may lead to birth defects(50). Statins inhibit Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the mevalonate pathway which converts HMG-CoA to mevalonic acid. Therefore, inhibition of this pathway by statins may lead to a wide range of defects.

2.8 Clinical studies of statins and teratogenicity

2.8.1 Atorvastatin

There is limited clinical evidence on the potential teratogenic effects of atorvastatin. Case reports and clinical trials from 1966 to 2004 were evaluated for information regarding the impact of various cholesterol lowering agents including atorvastatin on both the mother and foetus. These case reports and trials were inconsistent and concluded that additional studies were needed to determine the overall safety of these drugs in pregnancy(9).

In a prospective, observational cohort study to examine a fetal toxicity risk of statins, 64 pregnant women taking statins and 64 comparison group women without exposure to known teratogens were followed up during pregnancy(51). The women in the statin group were exposed to atorvastatin \((n = 46)\), simvastatin \((n = 9)\), pravastatin \((n = 6)\), or rosuvastatin \((n = 3)\) during the first trimester. There was no difference in the rate of major malformations between the statin group \((1/46 \text{ live birth: 2.2\%})\) and the comparison group \((1/52 \text{ live birth: 1.9\%}, \ p = 0.93)\) (51).

In most studies done to evaluate the foetal outcomes following maternal exposure to atorvastatin and other statins the difference in the rate of major birth defects between the statin exposed groups and control was small and statistically non-significant. Similarly, there
was no statistical differences in the rates of miscarriages and birth weigh between women who received atorvastatin and women who did not receive atorvastatin during pregnancy in another study(52).

### 2.8.2 Lovastatin

A post-marketing surveillance study reported on 48 cases with known outcome of women using lovastatin during pregnancy (10). In this study by Manson et al, there were three (6.3%) spontaneous abortions, one (2.1%) stillbirth, four (8.3%) infants with congenital anomalies (atrial or ventricular septal defects, cerebral dysfunction, VATER complex, spinal bifida, and holoprosencephaly), 39 (81.2%) normal outcomes, and one case of pedal oedema. The authors concluded that based on the timing of exposure and diversity of malformations, there was likely no causal relationship between taking the drug and congenital anomalies(10).

### 2.8.3 Simvastatin

Post-marketing surveillance data are available on 86 cases with known pregnancy outcome following exposure to simvastatin(10). Among these cases, there were (15.1%) spontaneous abortions, (1.2%) fetal death, (5.8%) congenital anomalies (polydactyly, unilateral cleft lip, hypospadias, trisomy 18, and clubfoot), (3.5%) miscellaneous adverse outcomes. While the number of prospective reports available for evaluation was only sufficient to rule out a three-to fourfold increase in the overall frequency of congenital anomalies, these proportions do not exceed what would be expected in the general population. Based on findings from this interim evaluation, there is no relationship between exposure to therapeutic doses of these agents during pregnancy and the occurrence of adverse pregnancy outcomes(10).

### 2.8.4 Summary of clinical evidence

Post market surveillance of fluvastatin and pravastatin has not confirmed any foetal abnormalities statistically significant to be linked with teratogenicity of these statins(53).
There is scarcity of data on rosuvastatin and pitavastatin teratogenic potential. The general risk of major congenital abnormalities from exposure to statins in pregnancy is similar to background risk of the population (12). No specific pattern of abnormalities was observed. Existing studies are small with limitation in their quality and as a result there is no evidence to demonstrate that use of statins in pregnancy increases the risk of foetal abnormalities (53).

2.9 Animal studies on statins

2.9.1 Atorvastatin

In a study by Dostal et al; the developmental toxicity of atorvastatin was investigated in pregnant rats and rabbits given daily oral doses during organogenesis (54). Rats received doses of atorvastatin ranging from 0 to 300mg/kg and rabbits received doses of atorvastatin
ranging from 0 to 100mg/kg. No teratogenic effects of atorvastatin were seen in rats and rabbits, even at maternally toxic doses. However, foetal body weight was lower than normal(54). Another study in rats showed that atorvastatin at maternally toxic doses resulted in a 45% lower survival rate of the offspring, decreased body weight, and abnormal neonatal development (55).

In a randomized-controlled animal study to determine the effects of atorvastatin on experimentally induced endometriosis in a rat model, rats in which endometriotic implants were induced were randomly divided into low and high dosages of atorvastatin(56). In this study, high dose atorvastatin caused a significant regression of endometriotic implants(56).

2.9.2 Lovastatin

In a study by Minks et al; administration of 800 mg/kg/day of lovastatin to rats from days 6 to 17 of gestation produced fetal malformations of the vertebrae and ribs in 29% of the litters, and there was a treatment-related increase in the incidence of gastroschisis. Lovastatin at 60 and 90 mg/kg/day also produced fetal malformations of the vertebrae and ribs(14). No drug-induced changes were seen in rabbits or mice given lovastatin at doses nine to 50 times the maximum recommended dose for humans

2.9.3 Fluvastatin

Oral administration of fluvastatin at 12 and 24 mg/kg/day to mated rats from day 15 of gestation through weaning resulted in maternal mortality at the time of parturition and during lactation ((57). Microscopic evaluation performed on a study involving dogs and monkeys on the toxic effects of fluvastatin reviewed significant cardiac myopathy on the dying
animals(58). Drug-related clinical signs, significant maternal body weight loss, and an increase in stillborn pups and neonatal mortality were also noted at one or both dose levels. No evidence of teratogenicity in rats or rabbits given high doses of fluvastatin were noted(57).

2.10 Methods used in teratogenicity testing

It is recommended that drugs should be examined for teratogenic activity in two species of animal. In most cases the species used are the rat or mouse and the rabbit. In Britain, three dose-levels are usually used in a teratogenic screen(59). In a three dose-level test, the high dose should be toxic but not lethal to the mother, the low dose should produce a clinically relevant effect and the third dose should be intermediate between the other two. Drugs should be given to test animals by the same route as they are to be administered clinically. If the drug is to be given orally, the best method of animal dosing is by gavage. There are disadvantages of administering drugs in the diet: firstly, it is difficult to estimate the exact amount of drug taken by the animal, and secondly, if the drug has an unpleasant taste, the animal will not eat the food(59).

Drug treatment should be started early enough, and continued long enough, to cover the period of organ formation in the species used for the test. Thus, in the rat and mouse treatment should extend from the 6th to the 15th days of pregnancy, inclusively, and in the rabbit it should extend from the 6th to the 18th days, inclusively. The time of mating should be estimated as closely as possible in order to determine the beginning of pregnancy. In the case of rodents, the males should only be caged with the females for a limited time: at the end of the mating period, successful copulation should be verified by the examination of a vaginal smear for spermatozoa. In the case of rabbits, we always mate the doe with two bucks and
then inject the doe intravenously with 25 IU chorionic gonadotropin. As far as possible, females of comparable weight and age range should be used in each test(59).

In some instances it may be advisable to use virgin females but in others females of known fertility are to be preferred. It is advisable to cage animals singly in order to avoid effects due to overcrowding. Females should be weighed regularly throughout pregnancy and daily during the dosing period. All animals should be examined daily. Careful handling should be practiced throughout and all clinical observations should be recorded. Vaginal bleeding should be noted, as this may be associated with resorption or abortion(59).

2.11 Study Rationale

Statins are in the category X group of drugs in pregnancy which makes them contraindicated in pregnancy yet they are the mainstay treatment option in hypercholesterolemia. Increasing evidence suggests that hypercholesterolemia during pregnancy initiates pathogenic events in
the foetus leading to increased risk of cardiovascular disease in the adult offspring (60). The FDA cited the lack of study data relating to the effects on a pregnant women and/or the foetus (as opposed to the existence of evidence of harm) as the main reason for putting the statins in this category (7). Animal studies have produced conflicting evidence on the potential teratogenicity of older statins. No animal studies have been done on developmental toxicity of the newer statins like rosvastatin and pitavastatin. Furthermore, no study has assessed the effect of statins of different doses within the therapeutic range on the birth weight of offspring. Studies in rats and rabbits failed to show a teratogenic effect of simvastatin (11). Studies in rats and rabbits have provided evidence that atorvastatin has developmental toxicity, but only at doses that induced maternal toxicity.

2.12 Aims and Objectives

2.12.1 Research Question

What are the developmental toxicity effects of rosvastatin and atorvastatin in time mated mice?
2.12.2 Aim
To determine the developmental toxicity effects of rosuvastatin and atorvastatin in time mated mice.

2.12.3 Specific Objectives
1. To determine the teratogenic effects of atorvastatin and rosuvastatin in time mated mice;
2. To determine the developmental toxicity of atorvastatin and rosuvastatin in time mated mice;
3. To compare the developmental and teratogenic effects of atorvastatin and rosuvastatin;
4. To compare the effects of Rosuvastatin and Atorvastatin on offspring birth weight in mice.

2.12.4 Hypothesis

Objective 1

H₀(1a): Atorvastatin has no teratogenic effect on time mated mice

H₀(1b): Rosuvastatin has no teratogenic effects on time mated mice

Objective 2

H₀(2a): Atorvastatin has no developmental toxicity effect on time mated mice

H₀(2b): Rosuvastatin has no developmental toxicity effect on time mated mice

Objective 3
H_0 (3): There is no difference in the developmental toxicity and teratogenic effects of atorvastatin and rosuvastatin in time mated mice.

**Objective 4**

H_0 (4): There is no difference in the offspring weights of time mated mice given rosuvastatin and atorvastatin in pregnancy.

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**CHAPTER 3: MATERIALS AND METHODS**

**3.1 Study Design**

An experimental design using time mated mice was conducted over a period of five (5) weeks.
3.2 Premating stage

Fifty six (56) mice were used in the experiment and they were divided into 7 groups of 8 mice each. The mice were kept under conditions of 12hrs darkness and 12hrs light and were fed adequately. Rosuvastatin was administered at doses of 10, 40, 100mg/kg/day via oral gavage route once every day for 7 days prior to the mating. Atorvastatin was administered at doses of 10, 40, 100mg/kg/day via oral gavage route once every day for 7 days prior to mating.

3.3 Mating Stage.

One male mouse was used for mating with 2 female mice from day 7 after introduction of the study drug. All the study groups and the control group started mating concurrently on day 7 following the beginning of study drugs administration. The mating duration was 10 days. After ten days of mating, the male mice were separated from the female mice and the female mice were all assumed to be pregnant.

3.4 Post-mating stage

Maternal body weight measurements were done prior to mating, on day 10 of mating and on day 17 (which was 7 days after the mating). Observation for morbidity and mortality and availability of food and water were done once every day throughout the study period. The wood shavings bedding were changed once every 2 to 3 days throughout the study period. Thorough clinical observation and recording was done daily in all the mice in the 6 study groups and the control group. The mice were allowed to carry their pregnancy to term and allowed a normal delivery.

3.5 Drug Administration
Rosuvastatin was administered at doses of 10, 40, 100mg/kg/day via oral gavage route once every day in each of the 3 rosuvastatin study groups a week prior to mating and throughout the gestation period. Atorvastatin was administered at doses of 10, 40, 100mg/kg/day via oral gavage route once every day in each of the 3 atorvastatin study groups from a week prior to mating and throughout the gestational period. The controlled group received distilled water via oral gavage route a week prior to mating and throughout the gestational period.

**Table 3.1:** Study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose mg/kg/day</th>
<th>Number of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Rosuvastatin</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Rosuvastatin</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Rosuvastatin</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Atorvastatin</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Atorvastatin</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>Atorvastatin</td>
<td>100</td>
<td>8</td>
</tr>
</tbody>
</table>

**3.6 Assessment of Offspring**

The mice were allowed to carrying their pregnancy to term and allowed a normal delivery. Offspring from each litter were weighed and underwent gross morphological examination.

**Table 3.2:** Gross morphological examinations

<table>
<thead>
<tr>
<th>Region</th>
<th>Gross morphological assessment specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>cranium</td>
<td>General formation of head (holoprosencephaly, anencephaly, microcephaly, encephalocele) eye bulge formation, ear bulge</td>
</tr>
<tr>
<td>Region</td>
<td>Examination</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Neck and trunk</td>
<td>General appearance</td>
</tr>
<tr>
<td>Vertebral column</td>
<td>General curvature of spine, meningomyelocele</td>
</tr>
<tr>
<td>Abdomen</td>
<td>General appearance of abdomen and assessment for the presence of gastroschisis and omphalocele</td>
</tr>
<tr>
<td>Limbs</td>
<td>Polydactyly, syndactyly, missing digits, extra digits, missing limbs, any other deformation on limbs</td>
</tr>
</tbody>
</table>

### 3.7 Statistical Analysis

Means and standard deviations for all measured parameters were calculated using the Statistical Package for Social Scientists (SPSS) Version 16, Chicago, USA. Statistical comparison of maternal and litter body weight were done using one-way analysis of variance (ANOVA). Where the assumptions of ANOVA were violated, Kruskal-Wallis test was used. Significance level for all statistical tests was set at alpha=0.05.

### 3.8 Ethical Approval

Ethical approval to carry out the research was granted by the Joint Parirenyatwa Hospital and College of Health Sciences Research Ethics Committee (JREC /132/14). The approval to use the animals was granted by the University of Zimbabwe Animal House. All the experiments done were in accordance with the ethical guidelines and standards on animal handling from the University of Zimbabwe Animal House.

### CHAPTER 4 RESULTS

#### 4.1 Maternal weight changes

#### 4.1.1 Atorvastatin and Control

Comment [SK18]:
The study groups were mated for 10 days. The baseline maternal weight of all mice before mating, on day 10 of mating and day 17 (day 7 post-mating) were recorded. A total of 18 out of 24 mice got pregnant in the atorvastatin groups while six of eight mice got pregnant in the control group.

**Table 4.1:** Maternal weight changes in atorvastatin and control groups

<table>
<thead>
<tr>
<th>Mean maternal weight/grams</th>
<th>Control</th>
<th>Atorvastatin 10mg/kg</th>
<th>Atorvastatin 40mg/kg</th>
<th>Atorvastatin 100mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>21.25 ± 1.64</td>
<td>24.51 ± 2.21</td>
<td>23.49 ± 2.10</td>
<td>23.13 ± 3.71</td>
</tr>
<tr>
<td>Day 10</td>
<td>25.62 ± 2.02</td>
<td>25.88 ± 2.18</td>
<td>24.85 ± 1.72</td>
<td>24.43 ± 4.47</td>
</tr>
<tr>
<td>Day 17</td>
<td>33.13 ± 4.20</td>
<td>34.12 ± 3.54</td>
<td>30.85 ± 3.98</td>
<td>33.41 ± 7.71</td>
</tr>
<tr>
<td>Weight gain</td>
<td>11.88 ± 3.03</td>
<td>9.61 ± 2.91</td>
<td>6.85 ± 3.19</td>
<td>10.28 ± 5.06</td>
</tr>
<tr>
<td>Percentage weight gain (%)</td>
<td>55.60 ±12.41</td>
<td>39.21 ±12.83</td>
<td>29.16 ±13.16</td>
<td>44.44 ±19.26</td>
</tr>
<tr>
<td>Number of pregnant mice</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

The table above shows the changes in weight from baseline of the pregnant mice in three atorvastatin study groups and the control group. A one way ANOVA was performed to test the hypothesis that the mean percentage weight gain of pregnant mice in the Atorvastatin and the Control groups were equal. There were statistically significant differences in the mean percentage weight gain between the atorvastatin groups and the control group \( F (df = 3, 19) = 3.458, p = 0.037 \). The Turkey multiple comparisons done at the 0.05 significance level found that the mean percentage weight gain for the control (mean = 55.60 ±12.41g, N = 6) was significantly higher than that of atorvastatin 40mg/kg (mean = 29.16 ±13.16, N = 6) (p = 0.024) but not significantly higher than that of atorvastatin 10mg (p = 0.234) and atorvastatin 100mg/kg (p = 0.553). The atorvastatin groups mean weights were not found to be significantly different from each other hence no relationship between maternal weight gain.
and the dose of atorvastatin. Figure 4.1 shows the mean percentage maternal weight changes during the study across the atorvastatin and control groups.

**Figure 4.1:** Maternal percentage weight changes in atorvastatin and control groups

### 4.1.2 Rosuvastatin and Control

A total of 16 out of 24 mice got pregnant in the rosuvastatin groups.

**Table 4.2:** Maternal weight changes in rosuvastatin and control groups

<table>
<thead>
<tr>
<th>Mean maternal weight/grams</th>
<th>Control</th>
<th>Rosuvastatin 10mg/kg</th>
<th>Rosuvastatin 40mg/kg</th>
<th>Rosuvastatin 100mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>21.25 ± 1.65</td>
<td>24.25 ±2.19</td>
<td>22.34 ±1.12</td>
<td>22.55 ±2.03</td>
</tr>
<tr>
<td>Day 10</td>
<td>25.62 ± 2.01</td>
<td>24.85 ±2.06</td>
<td>25.36 ±1.62</td>
<td>24.23 ±1.66</td>
</tr>
<tr>
<td>Day 17</td>
<td>33.13 ± 4.20</td>
<td>34.09 ±4.21</td>
<td>31.21 ±4.25</td>
<td>31.17 ±3.30</td>
</tr>
<tr>
<td>Weight gain</td>
<td>11.88 ± 3.03</td>
<td>9.84 ±2.58</td>
<td>8.87 ±4.00</td>
<td>8.62 ±1.27</td>
</tr>
<tr>
<td>Percentage weight gain</td>
<td>55.60± 12.41</td>
<td>40.58±9.72</td>
<td>39.70±18.46</td>
<td>38.23±2.18</td>
</tr>
<tr>
<td>Number of pregnant mice</td>
<td>6</td>
<td>8</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>
The table above shows the changes in weight from baseline of the pregnant mice in three rosvastatin study groups and the control group. A one way ANOVA was performed to test the hypothesis that the mean percentage weight gain of pregnant mice in the rosvastatin and the Control groups were equal. There were no statistically significant differences between the mean percentage weight gain between the rosvastatin groups and the control group \([F (df = 3, 19) =2.332, p = 0.113]\). The Turkey multiple comparisons done at the 0.05 significance level found that there was no significant differences in the percentage weight gain between the control group and the rosvastatin groups and no difference between the rosvastatin groups when they were compared to each other (all \(p\) values > 0.05). Figure 4.2 shows the mean percentage maternal weight changes across the rosvastatin and control groups.

**Figure 4.2**: Maternal percentage weight changes in rosvastatin and control groups

### 4.1.3 Summary of mating period

A total of 40 out of the 56 female mice got pregnant. One miscarriage was recorded in the rosvastatin 100mg/kg group. A total of six deaths occurred throughout the experiment period, three from rosvastatin 100mg/kg group, two from atorvastatin 100mg/kg group and one from the control group. The average number of pregnant mice per group in the entire study was five.
4.2.1 Pregnancy Outcome

The mice were allowed to carry their pregnancy to term and delivered vaginally. A total of 244 offspring were delivered by 40 mice. Table 4.3 below shows the number of offsprings, miscarriages, and the mean litter size per study group.

Table 4.3: Comparison of Litter size number of offsprings and miscarriages

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of offspring</th>
<th>Miscarriages</th>
<th>Mean litter size ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33</td>
<td>0</td>
<td>5.50 ± 3.21</td>
</tr>
<tr>
<td>Atorvastatin 10mg</td>
<td>42</td>
<td>0</td>
<td>7.00 ± 2.77</td>
</tr>
<tr>
<td>Atorvastatin 40mg</td>
<td>34</td>
<td>0</td>
<td>5.67 ± 1.86</td>
</tr>
<tr>
<td>Atorvastatin 100mg</td>
<td>42</td>
<td>0</td>
<td>8.40 ± 1.34</td>
</tr>
<tr>
<td>Rosuvastatin 10mg</td>
<td>53</td>
<td>0</td>
<td>6.62 ± 2.67</td>
</tr>
<tr>
<td>Rosuvastatin 40mg</td>
<td>25</td>
<td>0</td>
<td>5.00 ± 2.24</td>
</tr>
<tr>
<td>Rosuvastatin 100mg</td>
<td>15</td>
<td>1</td>
<td>7.50 ± 0.71</td>
</tr>
</tbody>
</table>

4.2.2 Atorvastatin vs. Control; Mean litter size

The table above shows the changes in mean litter mice in six study groups and the control group. A one way ANOVA was performed to test the hypothesis that the mean litter size of pregnant mice in the atorvastatin and the control groups were equal. There were no statistically significant differences between the mean litter sizes between the atorvastatin groups and the control group \([F (df = 3, 22) =1.645, p = 0.212]\). Figure 4.4 shows the mean litter size changes across the atorvastatin and control groups.
4.2.3 Rosuvastatin vs. Control; Mean litter size

A one way ANOVA was performed to test the hypothesis that the mean litter size of pregnant mice in the rosuvastatin and the control groups were equal. There were no statistically significant differences between the mean litter sizes between the rosuvastatin groups and the control group [F (df = 3, 20) =0.657, p = 0.590]. Figure 4.5 shows the mean litter size changes across rosuvastatin and control groups.

4.2.4 Mean offspring weight Atorvastatin and Control

A total of 118 offsprings were delivered, eight of which were removed from the study because they were inexaminnable (they were macerated by mice who were eating some of their offspring). Table 4.4 and figure 4.6 show the mean offspring weights of atorvastatin and control groups.

Table 4.4: Mean offspring weight in atorvastatin and control groups in grams

<table>
<thead>
<tr>
<th>Experimental group/grams</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
<tr>
<td>Atorvastatin 10mg</td>
<td>34</td>
<td>1.3044</td>
<td>0.08313</td>
<td>0.01426</td>
<td>1.2754</td>
</tr>
</tbody>
</table>
A one way ANOVA was performed to test the hypothesis that the mean birth weight of offspring in the Atorvastatin and the Control groups were equal. Multiple comparisons were performed using the Turkey and Bonferroni tests. The mean birth weight of offspring was found to be different across the Atorvastatin and Control groups \( F(3, 135) = 6.612, p < 0.001 \).

Turkey multiple comparison performed at 0.05 significance level found that there was no significant difference between the mean offspring birth weight of the control (mean weight = 1.3614, SD = 0.17046, N = 29), atorvastatin 10mg/kg (mean weight = 1.3044, SD = 0.08313, N = 34), atorvastatin 40mg/kg (mean weight = 1.2474, SD = 0.26124, N = 34) and

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Sample Size</th>
<th>Mean Birth Weight</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>F (df=3, 135)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin 40mg</td>
<td>34</td>
<td>1.2474</td>
<td>0.26124</td>
<td>0.04480</td>
<td>1.1562</td>
<td>1.3385</td>
</tr>
<tr>
<td>Atorvastatin 100mg</td>
<td>42</td>
<td>1.4181</td>
<td>0.14046</td>
<td>0.02167</td>
<td>1.3743</td>
<td>1.4619</td>
</tr>
<tr>
<td>Control</td>
<td>29</td>
<td>1.3614</td>
<td>0.17046</td>
<td>0.03165</td>
<td>1.2965</td>
<td>1.4262</td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>1.3367</td>
<td>0.18465</td>
<td>0.01566</td>
<td>1.3057</td>
<td>1.3677</td>
</tr>
</tbody>
</table>

**Figure 4.5:** Mean offspring weight distribution in atorvastatin and control groups
atorvastatin 100mg/kg (mean weight = 1.4181, SD = 0.14046, N = 42). However, there was statistically significant difference in the mean birth weights between mice administered atorvastatin 100mg/kg vs. atorvastatin 10mg/kg (p = 0.027), and atorvastatin 100mg/kg vs. atorvastatin 40mg/kg (p = 0.001). The Bonferroni test confirmed the results found by the Turkey post-hoc test.

A Kruskall Wallis test was used to test for differences between the mean weights of offspring because the data was not normally distributed. The Kruskall Wallis test for comparison of mean offspring weight in the control and atorvastatin groups indicated that there was a statistically significant differences in the distribution of the offspring weight between the groups \( \chi^2(3) = 25.6 \) and \( p < 0.001 \).

### 4.2.5 Rosuvastatin Offspring

A total of 93 offsprings were delivered weighed and examined from the rosvastatin study groups. The table 4.5 and figure 4.7 below shows a comparison of the mean weight of the rosvastatin and the control groups.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
</tbody>
</table>

Table 4.5: Offspring Mean Weight Rosuvastatin and Control
<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SEM</th>
<th>CI</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rosuvastatin 10mg</strong></td>
<td>53</td>
<td>1.3606</td>
<td>0.14739</td>
<td>0.02025</td>
<td>1.3199</td>
</tr>
<tr>
<td><strong>Rosuvastatin 40mg</strong></td>
<td>25</td>
<td>1.2360</td>
<td>0.17364</td>
<td>0.03473</td>
<td>1.1643</td>
</tr>
<tr>
<td><strong>Rosuvastin 100mg</strong></td>
<td>15</td>
<td>1.3447</td>
<td>0.13464</td>
<td>0.03476</td>
<td>1.2701</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>29</td>
<td>1.3614</td>
<td>0.17046</td>
<td>0.03165</td>
<td>1.2965</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>122</td>
<td>1.3333</td>
<td>0.16317</td>
<td>0.1477</td>
<td>1.3040</td>
</tr>
</tbody>
</table>

**Figure 4.6:** Mean offspring weight distribution in rosuvastatin and control groups.
A one way ANOVA was performed to test the hypothesis that the mean birth weight of offspring in the rosuvastatin and the control groups were equal. Multiple comparison using the Turkey and Bonferonni test was then done.

The mean birth weight of offspring was found to be different across the groups \([F (df = 3, 118) = 4.052, p = 0.01]\) Turkey Multiple comparison performed at 0.05 significance level found that the mean birth weight for the control group. (Mean = 1.3614, SD = 0.17046, N = 29) was significantly higher than that of rosuvastatin 40mg/kg (mean = 1.2360, SD = 0.14739, N = 25) \((p = 0.022)\) but not significantly different from rosuvastatin 10mg/kg (mean =1.3606, SD = 0.14739, N = 53) \((p = 1.000)\) and rosuvastatin 100mg/kg (mean = 1.3447, SD = 0.13464, N = 15) \((p = 0.987)\). The mean birth weight for rosuvastatin 10mg/kg (mean =1.3606, SD = 0.14739, N = 53) was significantly higher than that of rosuvastatin 40mg/kg \((mean = 1.2360, SD = 0.14739, N = 25) (p = 0.008)\) and not significantly different from that of rosuvastatin 100mg/kg \((mean = 1.3447, SD = 0.13464, N = 15) (p = 0.986)\). However there was no significant difference the mean birth weight in the rosuvastatin 40 and 100mg/kg groups \((p = 0.154)\).

Bonferonni Multiple comparison performed at 0.05 significant levels also confirmed the same results shown by Turkey test that there was a significant difference in the offspring birth weights of the rosuvastatin 40mg/kg and control \((p = 0.025)\) groups but no significant difference between the Control and the rosuvastatin 10mg/kg \((p = 1.000)\) and 100mg/kg \((p =1.000)\). A significant difference was also noted in the mean weight of rosuvastatin 10mg/kg and 40mg/kg \((p = 0.009)\).

A Kruskall Wallis test was used to test for differences between the mean weights of offspring because the data was not normally distributed. The  Kruskall Wallis test for comparison of
mean offspring weight in the control and rosuvastatin groups indicated that there is statistically significant differences in the distribution of the offspring weight between the groups, \( \chi^2(3) = 12.1 \) and \( p < 0.007 \).

### 4.3 Gross Morphological Examination

A total of 207 offspring underwent gross morphological examination. Of the 244 offsprings, 37 of the offspring were not examinable (there were macerated secondary to mutilation by the maternal mice who were eating their offspring). All the mice were examined for gross anatomical abnormality specifically looking for the following birth defects: general formation of the head, holoprosencephaly, anencephaly, microcephaly, encephalocele, eye bulge formation, ear bulge formation and the general appearance of the neck and trunk. In the vertebral column the presents of spinal bifida and general curvature of the spine was assessed in all offspring. The abdomen was examined for gastroschisis and omphalocele. The limbs were assessed for polydactly, syndactly, missing digits, extra digits, missing limbs, and any other limb deformities.

On gross morphological examination in all the 207 offspring from the seven study groups, no gross morphological deformities or abnormalities were observed.

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**CHAPTER 5: DISCUSSION AND CONCLUSIONS**
5.1 Discussion

The purpose of this study was to determine the developmental toxicity of different therapeutic doses of atorvastatin and rosuvastatin in a pregnant mouse model. The study also assessed the changes in foetal birth weight and examined offspring for gross morphological abnormalities.

Maternal weight gains were significantly lower in the atorvastatin study groups (between 29% and 45%) compared with the control group (60%). This could have been due to the physiological response of the mice following introduction of a xenobiotic. Another possible explanation of lower maternal weight gain changes in the atorvastatin drug related toxic effects of the statins. However, there was no relationship between the percentage weight gain and the dose of the statin as there was greater percentage of weight gain in atorvastatin 100mg/kg (44.44%) compared to the 40 and 10mg/kg groups (29.16% and 39.21%). Only the atorvastatin 40mg/kg had a significant difference in weight gain compared to the control.

These findings are consistent with the study conducted by Dostal et al. (1994) (54). In the study by Dostal et al, atorvastatin administered to rats at 0, 10, 100 and 300mg/kg showed a decrease in maternal body weight gain and food consumption (43% and 23%, respectively) in the 300mg/kg treatment group (54). Similar finding were also noted by Henck et al. (1998), (55) in a study to investigate effects of atorvastatin on various aspects of reproduction and development at 0, 20, 100, 225mg/kg. Maternal toxicity, characterized by morbidity/mortality (13%), reduced body weight gain and food consumption (% not reported), and pathologic lesions in the non-glandular mucosa of the stomach, occurred at 225 mg/kg (55). From these findings it can be noted that maternal toxicity effects of atorvastatin were noted in the dose above 100mg/kg which was also consistent with the findings in this study as there was no dose related effects of the drug on the maternal weight.
There was no significant difference between the percentage weight gain in the control and all the rosuvastatin groups (p = 0.113). This could mean that the maternal and developmental toxicity effects of rosuvastatin could be higher than 100mg/kg but there is need for further study on the maternal toxicity effects of rosuvastatin as the sample size in this study was small hence unable to make conclusions based on these results alone.

Thorough daily examination of the pregnant mice was done to assess for possible developmental toxicity of the statins. Only one miscarriage was noted in the rosuvastatin 100mg/kg group. No miscarriages were recorded in the atorvastatin and control groups at all the dose levels. From these finding it can be concluded statins were not found to be associated with increased risk of abortions pregnant mice. Similarly in the study by Henck et al. (1998), no abortions were recorded in rats at 225mg/kg atorvastatin. In the Dostal et al. study only one animal had total litter resorption at 300mg/kg atorvastatin (54).

A total of six maternal deaths occurred throughout the experimental period. The death rate was highest in the highest doses of atorvastatin (two) and rosuvastatin (three) compared to the control group (one). The maternal death rate was three times more in the rosuvastatin group compared with the control group. These results could mean that the rosuvastatin is associated with some degree of maternal toxicity at a dose of 100mg/kg or higher in mice. Faqi et al. (2012), investigated the developmental toxicity effects of PPD10558 a statin structurally similar to atorvastatin in rats and rabbits. No maternal death or toxicity were noted in rats at doses of up to 320mg/kg. In rabbits, marked maternal toxicity including mortality (eight deaths; 1 dose at 25 and 7 at 50 mg/kg/day), abortions (2 at 25 mg/kg/day and 6 at 50 mg/kg/day(15).

The mice were allowed to carry their pregnancy to term and delivered vaginally. The mean litter size were found not to be significantly different from the control in both the atorvastatin
(p = 0.212) and the rosuvastatin groups (p = 0.590). This is consistent with the study by Dostal et al. (1994) who observed that treatment of rats with atorvastatin up to 300mg/kg had no effect on live litter size or sex ratios(54).

Rosuvastatin had a significant effect on the offspring birth weight at a moderate dose of 40mg/kg (p = 0.025) while low and high doses had no effect on birth weight. This could be attributed to the lower average baseline weight of the maternal mice in the group which made them more susceptible to the direct toxic effect of the statins rather than their developmental toxicity effects as this trend was not observed in the animals exposed to the high dose of rosuvastatin 100mg/kg. If rosuvastatin had negative effect on offspring birth weight this trend should have been observed in the highest dose of rosuvastatin.

There was no significant difference in mean offspring birth weight between control group and the individual atorvastatin groups though there was significant differences in the comparison of the atorvastatin groups to each other. There was no dose depend decrease in mean weight in the atorvastatin groups with the atorvastatin 100mg/kg with the highest mean weight meaning the difference in the offspring weight were a result of the drug toxicity effects which were more depended on the individual animals not the amount of drug given.

Based on gross morphological assessment both atorvastatin and rosuvastatin had no developmental toxicity or teratogenic effects. Similarly, an embryo foetal development study by Henck et al. (1998) showed that atorvastatin had no maternal or developmental toxicity at 10 or 100 mg/kg/day in rats. However, pre- and postnatal administration of oral doses of up to 225 mg/kg/day of atorvastatin to female rats produces developmental toxicity in the presence or absence of maternal toxicity(55). Dostal et al (1994) concluded that atorvastatin at 10 mg/kg and 100mg/kg had no maternal or developmental toxicity in rats and rabbits (54).
In rabbits, marked maternal toxicity (7 deaths, body weight loss during and after treatment, and decreased food consumption) and abortion occurred at 100 mg/kg.(54) These findings are also consistent with the study by Faqi et al. (2011) which showed no maternal and developmental toxicity of PPD10558 at doses 0, 20, 80, or 320 mg/kg/day from gestation day 6 to 17 used in a rat model study(15). PPD10558 is a statin structurally similar to atorvastatin which is under development for treatment of hyperlipidemia.

However, Lankas et al.(2004) showed that lovastatin inhibitor induced foetal anomalies at a maternally toxic dose of 800 mg/kg/day in rats(61). This was contrary to the post market surveillances reports on lovastatin and simvastatin which showed no adverse outcome with both drugs(10). The no-effect level for developmental toxicity of lovastatin was 80 mg/kg/day, approximately 50–fold the highest recommended therapeutic dose of 80 mg/day(61). Furthermore the original developmental toxicity studies of lovastatin were re-examined and an alternative hypothesis developed for the mechanism of the previously observed rat developmental toxicity. It was hypothesized that the observed developmental abnormalities induced in rats by high dosages of lovastatin are secondary to the marked maternal toxicity that is produced early in gestation(62). Since this maternal toxicity is primarily due to the well-characterized, pharmacologically mediated effect of statins on the rat non glandular stomach(62), that is readily reversible with mevalonate, this hypothesis is consistent with the original observation of reversibility of the fetal abnormalities by mevalonate co-administration(14). From these finding there is no direct link between teratogenicity and statins without maternal toxicity.

5.2 Conclusions
Atorvastatin and rosuvastatin had no developmental toxicity effects in terms of offspring weight and occurrence of miscarriages. In addition, no teratogenic effects were observed with both drugs. From these finding and in literature there is no direct link between teratogenicity and statins without maternal toxicity as the teratogenic finding in lovastatin which is the only statin linked with birth defects in animal studies were at maternal toxic doses. Based on evidence from this study statins were not found to be associated with developmental toxicity including teratogenicity at non maternal toxic doses in animals. Needless to mention that finding in animals do not translate to the same effect in humans though there is concurrence with observed trends in humans as evidence from post market surveillance had shown no teratogenicity in humans , which makes the teratogenic potential risk of statins lower than what was previously thought..

5.3 Limitations

Caesarean section would have been the ideal method of delivery so that the offsprings would be delivered on the same day. Allowing the pregnancy to be delivered vaginally resulted in bias in birth weight as some offspring would have a high birth weight due to longer gestational period.

A control group with a known teratogenic drug would have made the comparison for the teratogenic effect more plausible.

5.4 Recommendation
There is need for further research to assess on the maternal toxic effects of the rosuvastatin in an animal model with a larger sample size.

REFERENCES


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