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Effect of Prenatal Tetracycline Hydrochloride administration on Skeletal Differentiation in Albino Rat fetuses(Rattus *novegicus*)

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Abstract: ***Objective:*** *To determine the effect of Tetracycline Hydrochloride on Albino rat fetuses skeleton differentiation at different dosages and gestations.* ***Study site:*** *Department of Human Anatomy.* ***Methods:*** *sexually mature 30 Albino rats weighing 150-200g were used upon conceiving. Dams were grouped into 1st, 2nd and 3rd trimester dosing groups each with 3 dams and the drug given via gastric gavaging daily at 9am in 5%DMSO as low dose 155mg/kg, medium dose 231.5mg/kg and high dose 310mg/kg beginning pregnancy day 0 for rats in trimester 1, day 8 for 2nd trimester rats and day 14 for 3rd trimester rats. Animals were sacrificed on day 20 of gestation, fetuses harvested and then killed with concentrated CO_2, dehydrated in 95% ethanol for 4 days then 100% ethanol for 4 days thereafter rinsed in distilled water, eviscerated and placed in 2% KOH solution for 24hours, rinsed then placed in 0.5% KOH solution containing Alizarin red for 24hours. After 24hours, the fetuses were drained and placed in 25% glycerol solution for 1 week then in 100%glycerol with a few drops of 0.5phenol solution. The skeleton was examined for skeletal differentiation and gross anomalies using Leica M205C Stereomicroscope mounted with DFC450C camera.* ***Results:*** *skeletal ossification was associated with dose levels across all trimesters.Trimester one, Ossification χ=17.32, p = 0.008, Trimester two and three χ= 24.95, p=0.001 and χ= 24.95, p=<0.001 respectively.*

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I. Introduction

Tetracyclines have been shown to have bone chelating effect with resultant poor cortical bone formation and altered epiphysial growth plate histogenesis in the fetus (1–3) which led to their initial categorization as category D medicines, meaning that they were not to be used during pregnancy(1,2,4,5). This categorization was based on studies done on the original molecule which were subsequently adapted for other tetracycline derivatives(5). Currently, there exists a controversy on the teratogenic effects of the different regimens of tetracyclines on the development of the fetal skeletal structures with some studies showing that tetracycline impairs bone morphogenesis and others do not(2,5,6). Recent studies on the other hand have shown that despite this categorization of tetracyclines in class D of the medicines that should not be used in pregnancy, not all tetracyclines affect bone histogenesis (2,5). Tetracyclines regimens are a group of broad spectrum antibiotics that are known to be very efficacious in treatment of atypical microbial infections and their benefits are being lost due to the existing controversy on teratogenicity(2). This study sought to determine the effect of tetracycline hydrochloride on Albino rat fetal skeleton differentiation at varied doses in different gestational periods.

II. Materials and Methods

Two hundred and forty Albino rat fetuses were were harvested on the 20th day of gestation from the 30 pregnant dams for the study. The 30 dams weighed between 150g-200g and were 10-12weeks of age. They were ovulating normally upon acquisition. 2 dams were placed with a fertile male Albino rat for mating purposes and was removed every morning at 0900hrs for 2 hours and a vaginal smear from the dam taken for pregnancy determination. A positive pregnancy was confirmed by observation of large cornified cells, numerous neutrophils on the smear and scattered epithelial cells. The pregnant dams were grouped into 1st, 2nd and 3rd

trimester dosing groups each with 3 dams.Tetracycline Hydrochloride was administered to pregnant female Albino rats beginning day one of pregnancy as determined by the Herbsuer method. The drug dosages were guided by the Human Equivalent Dose(25-30mg/kg) from which the Animal Equivalent Dose(155mg/kg-310mg/kg) was calculated. The Animal Equivalent Doses were administered as a solution of Tetracycline hydrochloride in 5%Dimethyle Sulphoxide once daily as follows: low dose group 155mg/kg, medium dose group 231.5mg/kg and high dose group 310mg/kg. Tetracycline hydrochloride was given via gastric gavaging daily at 9am to the pregnant dams beginning day 0 of pregnancy for rats in trimester 1, day 8 of pregnancy for the 2^{nd} trimester rats and day 14 for the 3^{rd} trimester rats. Animals were sacrificed on the 20^{th} day of gestation and a Caeserian section done. The harvested fetuses were then killed with concentrated CO2 and dehydrated in 95% ethanol for 4 days then 100% ethanol for another 4 days. Thereafter they were rinsed in distilled water and eviscerated then placed in 2% KOH solution for 24hours after which they were rinsed and placed in placed in 0.5% KOH solution containing Alizarin red for 24hours. After 24hours the fetuses were drained and placed in 25% glycerol solution for 1 week thereafter in 100%glycerol with a few drops of 0.5phenol solution for preservation. The thoracic cage and the appendicular skeleton were then examined for evidence of skeletal differentiation.

Statistical Analysis

The data was analyzed using SPSS and Excel statistical software. The study compared how the three dose levels (Low, Medium and High) and control compared in the three trimesters (Trimester 1, Trimester T2 and Trimester 3) with regards to skeletal differentiation. To determine the significance, Chi-square test was used and 5% significance level ($\alpha = 0.05$) was assumed. The results were considered to be significant whenever the probability value was less than 0.05 ($p<0.05$). The results are presented below.

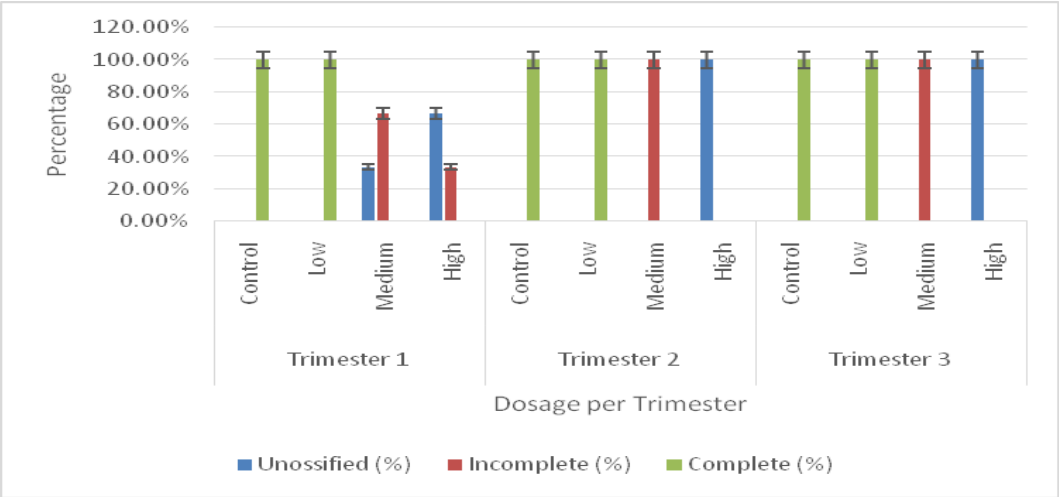
III. Results

Comparison of dosage in trimesters 1, 2 and 3 for ossification

Ossification status was analyzed using cross tabulations and Chi-square tests. This tested whether the dosage was significantly associated with Ossification status in the experimental rats. The results are as in Table 1. The results revealed that in Trimester one, Ossification was associated with dose levels, χ=17.32, p = 0.008. In Trimester two and three, this was found to be the case, χ= 24.95, p=0.001 and χ= 24.95, p=<0.001 respectively.

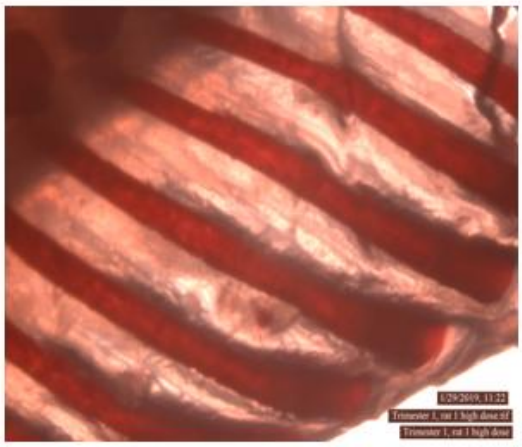
Table no 1: Effect of dosage (control, low, medium and high) on Ossification

Trimester	Dose Categories	Ossification			χ (p-value)
		Unossified(%)	Incomplete(%)	Complete(%)	
Trimester 1	Control	0 (0.0)	0 (0.0)	3 (100.0)	17.32 (0.008)
	Low	0 (0.0)	0 (0.0)	3 (100.0)	
	Medium	1 (33.3)	2 (66.7)	0 (0.0)	
	High	2 (66.7)	1 (33.3)	0 (0.0)	
Trimester 2	Control	0 (0.0)	0 (0.0)	3 (100.0)	24.95 (0.000)
	Low	0 (0.0)	0 (0.0)	3 (100.0)	
	Medium	0 (0.0)	3 (100.0)	0 (0.0)	
	High	3 (100.0)	0 (0.0)	0 (0.0)	
Trimester 3	Control	0 (0.0)	0 (0.0)	3 (100.0)	24.95 (0.000)
	Low	0 (0.0)	0 (0.0)	3 (100.0)	
	Medium	0 (0.0)	3 (100.0)	0 (0.0)	
	High	3 (100.0)	0 (0.0)	0 (0.0)	

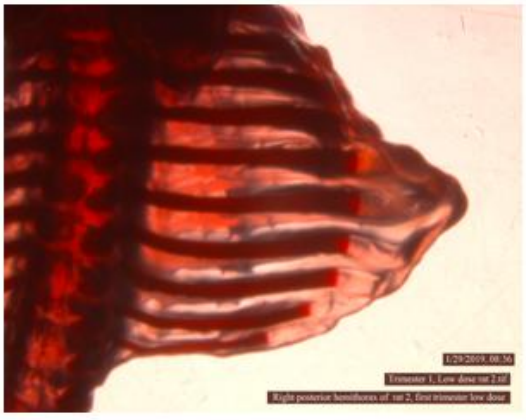


Graph 1: Effect of dosage (control, low, medium and high) on Ossification

Control group



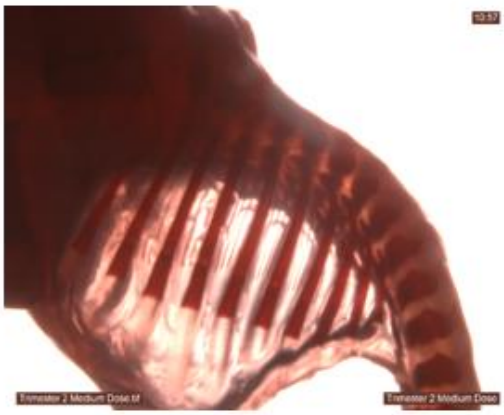
High dose group Trimester 1



Low dose group Trimester 1

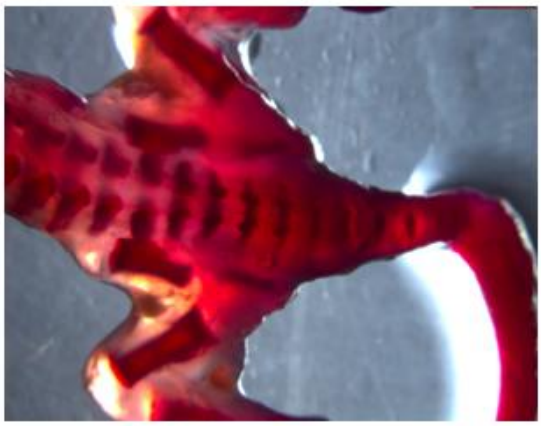
Low dose group Trimester 2

Medium dose group Trimester 1

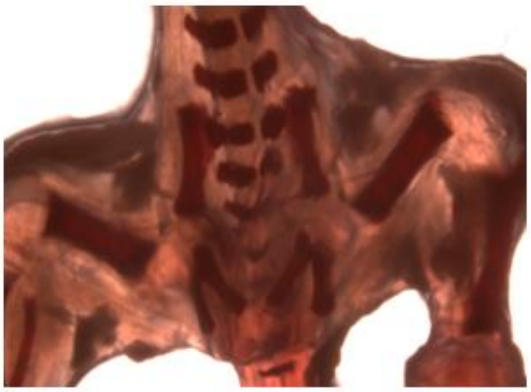


Medium dose Trimester 2

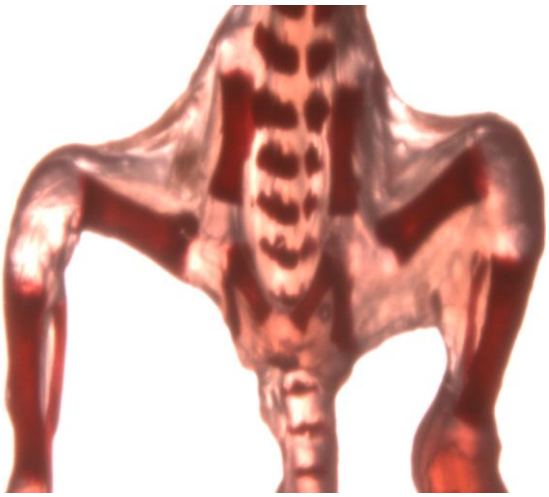
High dose group Trimester 2



Low dose group Trimester 3



High dose group Trimester 3



Medium dose group Trimester 3

IV. Discussion

Tetracycline antibiotics have been widely used in treatment of various bacterial diseases but since the recognition of their bone chelating properties, their use declined both in the paediatric population and pregnant women(1,2,5,6). Recent data shows tetracyclines to induce osteoblasts formation and inhibit osteoclast formation and function(1) and doxycline has been recommended for use in pregnancy and children following studies that showed it not to be bone teratogenic(1,2,5). Ossification is the process of laying down new bone by osteoblasts(7)

The study results revealed that ossification was associated with dose levels across all trimesters. Trimester 1 χ=17.32, p = 0.008, Trimester 2 χ= 24.95, p=0.001 and Trimester 3 χ= 24.95, p=<0.001. Low doses of tetracycline have been shown to propagate osteoblast proliferation and enhance osteod mineralization(1,8). In a study done on diabetic male DBA/2J mice upon long term exposure to doxycycline which is a Tetracycline, serum Procollagen type 1 N-terminal propeptide(P1NP) which is an indicator of bone development was found to be high in DBA/2J mice treated with doxycline(p=0.04), this implied an enhanced osteoblast activity with doxycycline treatment(9). Osteoclasts are the major cells that play a role in bone resorption and have their differentiation and maturation being regulated by osteoblasts which produces the Receptor Activator of Nuclear factor Kappa-B Ligand(RANKL) that is involved in osteoclast formation, function, as well as survival and Osteoprotegerin(OPG) that inhibits RANKL(10–13). Osteoclast precursors and mature osteoclasts poses the Receptor Activator of Nuclear factor Kappa-B(RANK) which is a binding site for RANKL. In another study, similar results on tetracyclines activity on bone were obtained and it was shown that doxycycline and minocycline as tetracyclines inhibit RANKL-induced osteoclast differentiation and maturation at low dosages(13). A study on ovarectimized rats showed an increase in bone trabecular area in rats administered with Tetracycline hydrochloride at low doses of 1.2 and 4.8mg/kg/day as compared to ovarectimized rats adiministered with a placebo which showed a decline in bone trabecular area as well as an increase in bone turnover. Similar results as in Tetracycline hydrochloride administered ovarectimized rats were obtained in ovarectimized estrogen treated rats which also showed an increase in bone trabecular area as well as an increase in trabecular number(14). In another study in a bone metastatic cancer mouse model, a combination of zolendronic acid and doxycycline reduced the total tumor burden by 74% as compared to zolendronic alone which reduced the tumor burden by 43% all compared to placebo(15). In the same study, doxycycline-zolendronic acid combination was shown to improve bone histomorphometric parameters(osteoid vlume, osteoid surface and population of osteoclasts per bone surface area)(15)

V. Conclusion

Despite Tetracyclines being known to be bone chelating, at low doses they have been shown to enhance bone formation through upregulating osteoblastic activity and inhibition of osteoclastic acitivity.

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