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A Study of the effect of Dexamethasone on the lung development and external morphology features during early embryonic stages in the swiss albino mice embryos *Mus masculus*

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Abstract

This study include the investigative the effect of Dexamethasone on the lung development and External morphology features at early Embryonic Developmental stages and evaluate Alpha Fetoprotein levels .Sixty pregnant mice were randomly divided into four groups and each group include 15 pregnant mice. Given the members of each group specific dose of (Dex) and at different time periods, while the control group injected with a solution of Normal Saline 0.9%, all animals received doses used by tail intravenous injection. In the end of specific period of pregnancy embryos were isolated from mothers ,mother's blood were taken to evaluate AFP levels .The results of statistical analysis at ($P \leq 0.05$) to different doses Dexamethasone show there a negative effects on mice embryo's body weights and the lengths increase with increasing of number and doses concentrations, (Dex) also affected on general external morphological features in embryos and on their lung development ,Dex has been affected on the lung development through lung cell proliferation and activity, especially at long terms and repeated doses.It's has been caused congenital malformations in embryos: Death of embryos ,placenta damage ,neural Tube Defect ,Trunk Torsion ,Head hemorrhage, Brain hypertrophy ,Liver Hypertrophy ,Letter C Shape Embryos, swelling ,Convoluted Tail ,Short limbs.The Altered in the AFP levels have been observed concurred with aberrant growth manifestations .

Key words: Dexamethasone, lung development, Albino Mice Embryos, AFP

دراسة تاثير عقار الديكساميثازون علي تكوين الرئة والخصائص المظهرية الخارجية خلال المراحل الجنينية المبكرة في اجنة الفئران البيضاء *Mus masculus*

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الخلاصة

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تضمنت الدراسة الحالية دراسة تأثير عقار الديكساميثازون على التكوين الجنيني للرئة والخصائص المظهرية الخارجية في المراحل الجنينية المبكرة وتقييم مستويات الالفا فيتو بروتين في عينات دم الامهات تم اخذها لتقييم الالفا فيتو بروتين. ستون فأرة حامل تم توزيعها عشوائياً الى اربع مجاميع كل مجموعة تضم خمس عشر فأرة حامل . اعطي افراد كل مجموعة جرعة محددة من الديكساميثازون وعلى فترات زمنية محددة بينما حقنت افراد مجموعة السيطرة بالمحلول الفسلجي 0.9% , تلقت جميع الحيوانات الجرع المستخدمة عن طريق حقن الوريد الذنبي حتى نهاية الفترات الزمنية المحددة. تم معاملة الحيوانات تحت الظروف نفسها وتم تحديد الجرعة بالاعتماد على وزن الجسم, نهاية كل فترة زمنية محددة تم عزل الاجنة من الامهات, عينات دم الامهات تم اخذها لغرض تقييم مستويات الالفا فيتو بروتين, نتائج جرع مختلفة من الديكساميثازون, اظهرت نتائج التحليل الاحصائي تحت مستوى احتمالية 0.05 لجرع مختلفة من الديكساميثازون تأثيرات سلبية على اوزان واطوال الاجنة ازدادت بزيادة عدد وتراكيز جرع الديكساميثازون اثر على على الخصائص المظهرية العامة في الاجنة وعلى تكوين الرئة.اثر الديكساميثازون على تكوين الرئة من خلال تكاثر ونشاط الخلايا الرئوية خاصة على المدى الطويل والجرعات المتكررة كما سبب تشوهات خلقية في الاجنة: موت الاجنة ,تلف المشيمة ,عيوب الانبوب العصبي ,انحراف الجذع, نزيف الرأس ,تضخم الدماغ ,تضخم الكبد , الاجنة بشكل حرف C , تورم ,التفاف الذيل ,قصر والاطراف.لوحظ ان التغير في مستويات الالفا فيتو بروتين يتفق مع مظاهر النمو الشاذ.

الكلمات المفتاحية : الديكسامثازون ,تطور الرئة ,اجنة الفئران البيضاء , الفافيتوبروتين

Introduction

Dexamethasone (Dex) a synthetic long action glucocorticosteroid hormone , is one of the most widely prescribed drug for the treatment an inflammatory disturbance such as adrenal hormone insufficiency , swelling , arthritis, redness of skin , asthma and kidney disorders. It is also been used to reduce the risk of neonatal respiratory distress syndrome (RDS). Glucocorticoids (GCs) play an important role in normal fetal development and are essential for the development and maturation of different fetal tissues including the liver, gut, adipose tissue ,skeletal muscle and lungs in preparation for extra-uterine life (Korgan *etal*.,2012). The fundamental function of respiratory system is the vital exchange of oxygen in the external environment with carbon dioxide in the cardiovascular system. The lung together with the trachea arises from the anterior foregut endoderm (Herriges and Morrisey, 2014). Mouse lung developmental stages can be divided into five overlapping stages :Embryonic stage (EMB)(9.5-E12.5),Pseudoglandular stage (PSG) (E13.5- E15.5), Canalicular stage (CAN) (E16.5-E17.5),Saccular stage (SAC)(E17.5- E19.5) and Alveolar stage (ALV)(P0-P18) (Beauchemin *etal*.,2016; Herriges and Morrisey, 2014).

During Embryonic stage and Pseudoglandular stage the two buds of lungs knuckle under a highly regulated branching process called Branching morphogenesis to generate a tree-like network of airways with thousands of terminal branches, Finally total alveolus maturations occurs during the Alveolarizzation stage, in all this stages of endodermal development lung mesenchym (meseoderm) development and interacts with lung endoderm to evolve branching, differenation and generat the various lineages with the lungs including airways ,pericytes and vascular smooth muscle (Herriges and Morrisey,2014).

Deformations represent mistakes in this process. The chemical bonding or translation genetic orders errors appear during the wildebeest for Abnormalities (Pastuszak, 2001). Congenital malformations in the fetus appears as a result of two factors: internal genetic causes result from mutations in a gene or chromosome abnormalities (Kraita *etal*.,2002) External factors (EFs) are environmental conditions experienced by the placenta and uterus which may lead to birth defects, (EFs) called Teratogen which changing the fetal growth (Alt,2000) it's include: radiation (Pastuszak,2001), chemicals like Methylmercury (Abdul Fattah, 2007; Alt,2000), drugs such as Cyclophosphamide (AlJawali, 2005) excessive smoking and alcohol , some virus infected parasites such as Syphilis, Toxoplasmosis, Rubella and ADIS ,A pathogenic microbes such as E.coli in Amniotic Fluid (Abdul Fattah,2007).Influence of deformed material as its concentration, and effect the resulting evolution of patients, are determined by the sensitivity and the generator as well as growing stage of

the target tissue (O'Day,2004), Because of the similarity between rodent and human in terms of fetal development and especially white mice and rat scientist focused their study on different technique, they often classify congenital malformations.

Materials and Methods

1-Experimental animals preparation: in this present study, Female Albino Mice, type *Mus masculus* the strain Balb /c ranged in age between 11 to 12 weeks , 30 ± 2 gm obtained from the Animal House belonging to the Biology Department - College of Education for pure science / Thi- Qar University , Mice were put in the room in plastic cages breeding with metal lids and Brush the cage with sawdust ,in the organization and controlled environmental conditions at the constant Photoperiod (12 hour day /12 hour night) cycle , ventilation, temperature ranged between 20-24 c° , The mice were took to the vet to ensure their health and they are free from disease. Mice were kept under cleanliness conditions of the cages through changing sawdust once every two days, Animals were given a sufficient amount of water and food from local source (Wheat 34% , barley 20% ,corn 25% ,animal protein 10% , powdered milk 10% ,salt 1% all the material were grinded and mixing with some oil and water until they become a paste coherent) (Tayfur,2013) and put in the designated place for the food in the cages, Animal breeding , then two mature females were caged together with one mature male overnight and in the following morning the females were checked for the vaginal plug (Saadalla,2009) , Date of mating was written on the cages ,the day of mating is Day zero (D_0) of pregnancy and the day after is the first day of pregnancy (Bogumil *etal.*, 2000).

2- Dexamethasone preparation.

Dexamethasone sodium phosphate (8mg /2ml) aqueous solution was used to treat the experimental animals in different doses. The mice were intravenously injected via tail vein. The different concentrations of drug were chosen according to therapeutic dose (8m to 70 kg) (Tayfur,2013) ,that equivalent to 0.1ml /1kg (0.002 ml /25 gm) from the mice weight. The experimental groups consisting of fifteen pregnant mice for each group, they were treated with different doses of drug as follows:

1-The first group: Was treated with the dose 0.05 mg for each 1kg from the body weight (Equivalent to0.001 mg for each 25 gm from the mouse body weight).

2-The second group: Was treated with the dose 0.1 mg for each 1kg from the body weight (Equivalent to0.002 mg for each 25 gm from the mouse body weight).

3-The third group: Was treated with the dose 0.2 mg for each 1kg from the body weight (Equivalent to 0.004 mg for each 25 gm from the mouse body weight).

The mice were injection starting from the eight day of gestation between the day and another. The mice were dissection at Embryonic days 11, 13 and 15 the embryos were isolated

3- Serum collection.

The pregnant mice were anesthetizatied by pieces of cotton wetted with chloroform in a glass jar before the animal stopping from movement blood was collected by using fine needle (1cc) via cardiac puncture, then the blood was separated by centrifugation 15 minutes at 3000 rpm then serum was taken and kept in plain tube at -20c.

4-Isolate of the mice embryos.

The pregnant mice were soaked in 70% ethanol to diminish the risk of contaminating the dissection with mouse hair. The skin was pinched and a small lateral incision was made at the midline with regular surgical scissors. The skin was Holed firmly above and below the incision and pulled apart toward the head and tail to expose the abdomen. The peritoneum was grasped with forceps and cut to expose the abdominal cavity. The uterine horn was removed by grasping the uterus below the oviduct and cutting it free along the mesometrium. Each embryo was separated by cutting between implantation sites along uterine horn. The muscular uterine lining was grasped by sliding forceps between the surrounding muscle layer and enveloped decidua tissue. The muscle layer was ridded and a portion of the decidua exposed then the embryo shelled out by using the tips of forceps. The length and weight of an embryo were noteed we examine the malformation and the changes and record it then take photograph by using camera photography for it , then embryos were kept in

container that contains 10% formalin until the start of the preparation of the histological sections .

5- Histological studies

The preparation of solutions and dyes, according to (Bancroft and Gamble, ,2008) .

1-The Fixation: The Samples (embryos) were fixated with formalin solution for 24 hours to keep all the size and arrangement of the cells and tissues samples which histological section prepare from it.

2-The Washing : The samples has been washed with the tap water for half an hour to remove the overload fixer from tissues.

3-The Dehydration : The samples were passed through a series ascending concentration of ethyl alcohol (35%,50%,70%, 80%, 90%, 95%, and 100%) for withdrawing water from the sample for a period of two hours for each concentration.

4--The Clearing :The samples were cleared with xylene for a full hour and on two-stages to make samples more clear.

5-The Infiltration :The samples were put in a mixture of wax melting point 56°C and xylene at ratio 1:1 and the mixture was put in the oven at temperature to 60 for 15 minutes afterward the samples were removed to the melted wax for half an hour each period.

6-The Embedding :The Samples were embedded in the molten wax paraffin by pouring the wax quietly in the iron template on the shape of the letter (L) ,the information written on paper (dose , embryonic day) and put at the side of the template , the samples left to cool and harden then they were removed from the template .

7 -The Trimming and Sectioning : Trimming of wax templates was done by a sharp knife then templates put on the installed holder on the microtome and the samples cut with a thickness of 5 µm, the tissue ribbon transferred to the water bath at temperature 37°C for the flatness , then tissue lifted from warm water on a glass slide marked by diamonds pen (Embryonic day and the dosage) after it has been wiped by Mayer's albumin and the slides put on the warm hot plate at temperature 40°C and then left to dry for 24 hours.

8- The Staining : The glass slides which contained the tissues were heated on the hot plate and then transferred directly to the xylene and left for half an hour for two stages to removed wax or wax remains then slides transferred to a series of descending concentration of ethyl alcohol (100%, 95%, 90%, 80%, 70%,50%,35%) two minutes for each concentration After that the slides were transferred to distilled water for two minutes, then the slides were put on Hematoxylin stain for 3 minutes , then the slides were washed with tap water until the blue color appeared after this slides put on Eosin stain for two minutes and then slides were passed on series of ascending concentration of ethyl alcohol (35%,50%,70%, 80%, 90%, 95%, 100%) for two minutes for each concentration the slides transferred to xylene twice for comment by text an hour each time.

9- The Mounting : D.P.X was used for mounting because of its quick drying, a drop of D.P.X Put directly on the tissue and then cover slide was put with the ensure of the absence of air bubbles, the slides were left to dry at temperature 40 C° on the hot plate for the purpose of speed drying process and put the slides in the slides box.

10- The Examination of Histological Slides and Photography.

The slides were examined by used Leica microscope, Germanic origin, in the different powerful zoom then photographed using a camera photography connected with the microscope.

6- The quantitative determination of mouse alpha-fetoprotein (AFP) concentrations in pregnant mice sera.

The pregnant mice Alpha Fetoprotein (AFP) concentrations were determinate with a sandwich-type enzyme linked immunosorbent assay (ELISA) according to the company manufacture, Elisa kits were obtained from MyBioSource (USA) .

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7- Statistical analysis

Data were presented as mean ± SE and analysis using SPSS software (version 24.0)for windows by Least Significant Difference Test (LSD) .Differences were considered statistically significant at $P \leq 0.05$.

Results

1-The effect of different doses Dexamethasone on external morphology features

The results of the present study showed the occurrence of many and the changes and phenotypic malformations in the embryos which were treated with different doses of dexamethasone, Such as small size (E11/0.05mg/kg Dex) (Fig1b) and a decrease in the weight of the embryos and the body of the fetus is convoluted to be like a ball or mass of meat (E11 /0.2 mg /kg Dex) (Fig2.a),a great damage that occur in placenta observed at (E11/0.1mg/kg Dex) (Fig 2b).

Malformation includes various regions for the fetus's body such as the head region like hemorrhage at E13 (all doses of Dex) (Fig3.b), Tumescence at the top of the head (Fig3.c) (E13/0.1 mg/kg Dex) E15(0.05mg/kg Dex)(Fig3d).)The curvature of the head towards the chest E15 (0.2 mg/kg Dex), spherical shape embryos (Fig4.b). In our results we observed The occurrence of curvature in the trunk at E11(0.2 mg /kg Dex)(Fig4c),E15(0.2mg /kg Dex) (Fig4.d) and with a deviation in the back bone with At caudal region of embryos there are various malformations occur such congestion in the end of tail convoluted in the tail and legs**.**

2- The effect of different doses Dexamethasone on the mice lung development.

Histological examination showed in the embryos at E11 there is a rapid development in the lung bud(0.05mg/kg Dex)(Fig6) (0.05 mg /kg Dex) to be like the flower(Fig7) this is a unique case record , At (0.2 mg /kg Dex)(Fig8) there is an elongation in the lung bud, there is a necrosis and hyperplasia in some epithelial cells (Fig 10and11)(0.05mg /kg Dex and 0.2mg/kg Dex), at E15(Fig 12) ,there is an advancing in branching morphogenesis process , in (0.1 mg / kg Dex), extensive branching of the distal epithelium and mesenchyme and the lung enter at pre canalicular stage (Fig13)(0.1mg/ kg Dex), resulting in formation of terminal sacs lined with epithelial cells ، a mucus hypersecretion in the lung at E15 (0.2mg/kg Dex)(Fig 14)**.**

3-The effect of different doses from dexamethasone on the level of alpha fetoprotein (AFP) .

The results of enzyme linked absorbent immune assay (Elisa) at this study showed that are many differences in the levels of alpha fetoprotein (AFP) concentrations in the pregnant mice sera, we use the level at control group at different days as a standard to be compared with another treatment groups, we noted the lower level of AFP concentration at embryonic day 11 (0.05 mg /kg Dex) was 0.05 ng/mg , while the higher AFP concentration was at embryonic day 13(0.05 mg /kg Dex). In the current study we found different AFP concentration values as (Table 3) explains. At E11 (0.05 mg / kgDex) AFP concentrations were very low and reduction in the body weight with rapid elongation in the lung bud, at (0.1 mg /kg Dex) AFP concentrations were very high as well as (0.2mg /kg Dex) resulting in death embryos ,advancing in lung maturation (the lung as flower appearance) ,At E13 (0.05mg /kg Dex)and (0.1 mg /kg Dex) AFP concentrations was very high embryos have hemorrhage in the head (HH) with loosely lung mesenchyme ,Neural Tube Defects (NTD),(0.2 mg/kg Dex). AFP concentrations was very low embryos have HH, advancing in lung maturation ,E15 (0.05mg /kg Dex)and(0.2 mg /kg Dex)AFP concentration very high with liver hypertrophy (LH) brain hemorrhage (BH) ,NTD, letter C shape embryos ,advancing in lung development ,elongation in epithelial air ways, (0.1 mg /kg Dex).

Discussion

During the first three months of pregnancy in human which that conforming the first week in mice pregnancy the cells are metamorphose and that is follow a special programmer in its growth (O'day,2004). The most critical time for any organ is

during its growth and formation of various structures (Gilbret, 2000), this period will be very sensitive to the changer factors thus it is named the critical period for organs and tissues (O'Day,2004) the changes are affected by growth stage and concentration of the material deformation (Pastuszak,2001). the reason of low weight (Table:1) comes from the great damage that occur in placenta this what we observed at (E11/0.1mg/kg Dex) (Fig2.b), this is what led to the diminishing of the exchange of nutrients materials between the mother and the fetus, thus reduced protein building process and this led to the birth an embryos with decreasing in the length(Table 2) and low weights compared with the control group. This is consistent with what's confirmed by (Siddiqui *etal.*,2013) during a study on pregnant female rats that has been treated with (4 mg / kg Dex).

At abdominal region we observed hypertrophy in liver region which clearly observed in earlier stages (E 13 and E 15) (Fig:3b and d) through the body wall

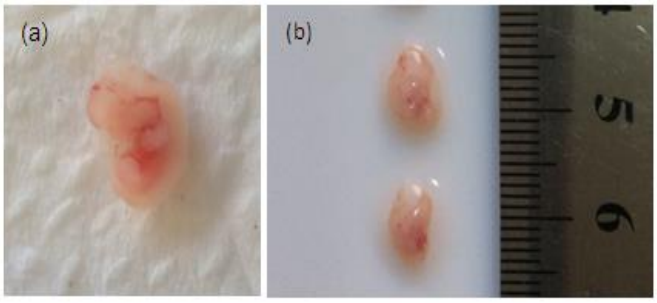

Figure 1: Embryos at embryonic day 11(a) control group, (b) Treatment with 0.1mg /kg Dex. Note the small size and different external morphology.

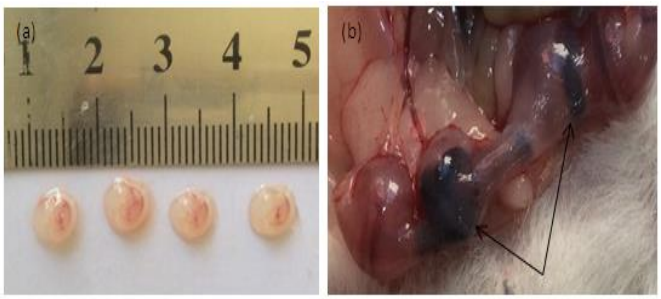

Figure 2: (a) Embryos at embryonic day 11(0.2mg/kg Dex), (b) show the uterus of pregnant mice treated with Dexamethasone 0.1mg /kg Dex.damage in the placenta (arrows)

Table 1: Show the effect of dexamethasone on mice embryos weight at different therapeutic doses and Embryonic days. *The mean difference is significant at the ($P \leq 0.05$) ,Mean± Std. Error, E: Embryonic day

Time /day	Treatment (Dexamethasone mg/kg)		
	(a)Groups	(b)Groups	Mean Difference (a-b)
E11	Control (0.00)	0.05	0.1 ± 0.11
		0.1	0.02* ± 0.11
		0.2	0.08* ± 0.11
E13	Control (0.00)	0.05	0.14* ± 0.03
		0.1	0.17* ± 0.03
		0.2	0.2* ± 0.03
E15	Control (0.00)	0.05	0.13* ± 0.09
		0.1	0.23* ± 0.09
		0.2	0.1 ± 0.09

(L.S.D 0.04).

Table 2 : Show the effect of dexamethasone on mice embryos lenght at different therapeutic doses and Embryonic days. *The mean difference is significant at the (P ≤0 .05) ,Mean± Std.Error. E: Embryonic day

Time /day	Treatment (Dexamethasone mg/kg)		
	(a)Groups	(b)Group	Mean Difference (a-b)
E11	Control (0.00)	0.05	0.1* ± 0.07
		0.1	0.2* ± 0.07
		0.2	0.3* ± 0.07
E13	Control (0.00)	0.05	0.1* ± 0.03
		0.1	0.14* ± 0.03
		0.2	0.14* ±0.03
E15	Control (0.00)	0.05	0.1* ± 0.04
		0.1	0.2* ± 0.04
		0.2	0.00 ± 0.04

(L.S.D 0.04).

In the current study, we administered a high dose of (Dex) large enough to cause fetal abnormalities or prenatal death. the exposure to (Dex) increased placental efficiency though reduced both fetal and placental weights , the exposure to (Dex) led to severe stress during an early stages of pregnancy on the fetus

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development and placenta this agree with (Lee *etal.*,2012).

Malformation in the head regions such as The curvature of the head towards the chest due to the curvature of the neck especially to form spherical shape embryos (Fig4b). This malformations explained with the defect in the nervous system, this agrees with (Lenoni *etal.*, 2013) when they confirmed that the treatment with (Dex) during the pregnancy period affected on the formation of synapses in the central nervous system and is this is what is elucidated by (Willmut *etal.*,1990) that any defect occur in any part in nervous system led to defect at another parts. the reason of brain hypertrophy is that the pore of neural plate isn't closed, because of the neural pore is closed in E9.5 - E10 (Rice andBarone,2000).we given the mice (Dex) before the period of neural plate closing that led to prevent closing it because of the Dex affected on embryos development, Most of nervous system defect comes from abnormal closing to the neural folds and called neural tube defect cases (NTD)(Al-hmoud *etal.*,2005).Take Dex during pregnancy especially at organogenesis cause defect in neural folds closing irregularly and led to (NTD) (Tayfour,2013) this agree with what we observed in current study .

The occurrence of curvature in the trunk and with a deviation in the back bone with a swelling this is consistent with what was observed by (Hamoudi,2005) who noticed the deformation in the trunk and deflection in the dorsal region of mice embryos when treated pregnant mice with 50mg /gm of paracetamol . Abdul majeed(1999).Explained the swelling in the dorsal region of fetus as resulting from rare malformation in spinal cord called Meningoen cephalo cell (MCC) As a result of the occurrence of deformation of the vertebrae leading to a swelling in the spinal cord on the dorsal surface under the body covered with skin ,Thus the (NTD) and deflection and curvature in the trunk led to formation of ball shape to embryos or spherical shape Embryos .

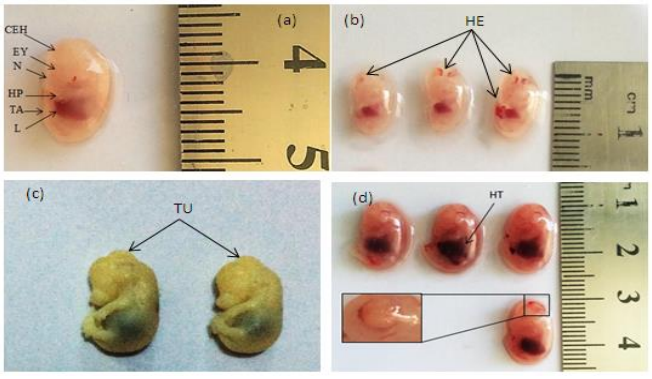

Figure 3: (a) E13 control, (CEH) Cerebral hemisphere, (EY) Eye, N: Nostril, (HP) Hand plate, (TA) Tail, (L) Liver,(b) E13 their mother was treated with dexamethasone (0.05 mg /kg Dex), (HE) Hemorrhage, (c). Embryos at E13 their mother was treated with Dex (0. 1 mg /kg Dex). (d)(TU) Tumescence in embryos' head .

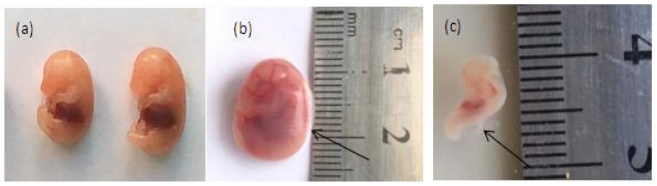

Figure 4: (a) Embryos at E15 control,(b)Embryos at E15 their mother treated with (0.2mg/kg Dex) .(b)Trunk torision(arrow) at E11(0.2mg/kg Dex).(c).E15 Trunk torision(arrow) (0.2mg/kg),(e) P1 (0.1mg/kg Dex) Trunk torision(arrows.

At abdominal region we observed hypertrophy in liver region which clearly observed through the body wall because of increasing hepatocyte size as a result of glycogen accumulation which what was concluded by Al khateeb *etal* (2014).The malformation in the retardation of limb formation this agree with Kim *etal* (2015) when they suggest that the prenatal which exposure to (Dex) in the mice induces fetal skeletal malformations. This may be belong to the effect of dexamethasone on limbs formation especially Forelimb development in the mouse commences at about E9.5 with the hind limb lagging behind by about half a day. Later developmental process is mainly just for growth and maturation of the component tissues converting, for instance, the miniature embryonic cartilage template into the bony skeletal elements of the adult limb (Martin, 1990). We treated the pregnant mice with dexamethasone at E8 thus it affected on limbs development.

Malformations at caudal region occur, This agrees with Tayfur(2013) and Copp *etal* they had (1994)explained that the delay of the closing the posterior neural pore is the main reason that leads to the

Table 3: Observ
embryonic days

tail torsion this delay causes stress at caudal bud because of the lack of balance between the tube neural and non-neural structure , and due to a reduction in the rate of cellular reproduction to the spinal cord (Martins, 1998) the exposure to prenatal Dex results in a placental defect as well as embryonic growth this is what has been confirmed by (Yun *etal*.,2016).

2- The effect of different doses Dexamethasone on the mice lung development.

Histological examination showed that the lung developing at E11 (Fig 5)the mice embryos begin as a simple epithelial tube surrounded by thick mesenchyme, this agrees with(Kim *etal*.,2015), because the treatment with Dex causes distorted branching morphogenesis process , this was confirmed by (Tuyl *etal*.,2001) .The mice lung appears as small bud at E9.5 (Beauchemin *etal*.,2002)from the ventral foregut endoderm grow into the surrounding splanchnic mesenchyme .the elongation in the lung bud (Fig6) this may be belong to effect of (Dex) on cellular proliferative activity for distal endodermal bud cells because there is just elongation to the bud without development to later development stage. At the pseudoglandular stage E13 (Fig9) lung is looking like a primitive small gland and it is the most active period of branching morphogenesis that leads to the formation of the conducting airways (Manwani,2009).At the pseudoglandular stage progresses the early pseudostratified epithelium is gradually replaced by columnar cells proximally and distally by cuboidal cells which it rich with glycogen, during pseudoglandular stage, all pre-acinar structures, including , pulmonary arteries and veins, pre-acinar airway, are formed (Joshi,2007). Necrosis and hyperplasia(Fig10 and 11) in some epithelial cells, belong to the rate of cell generation in embryo is faster than in the adults , thus any effect on the cell replication certainly affected on the genes it may be caused defect on new formed cells ratio therefor different area of embryo proliferation in different speeds and times during the organogenesis process , another reason that the alteration in biosynthesis led to structural and functional malformations because the alterations in DNA led to defects in protein synthesis resulting in alterations in functional and structural components this may be prevent the tissue differentiation and cause defect in dysgenesis , this is what cause embryos tissues lesions (Francis,1994).

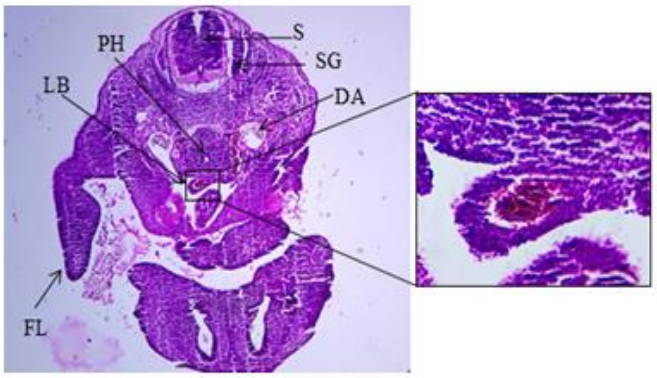

Figure5: Transverse section show embryos at embryonic day 11(40x) (LB) Lung bud (S)Spin (SG) Spinal ganglion (DA) Dorsal aorta (PH) Pharynx (C) Chorda (FL) Fore limb (HandE) magnificent section show lung bud(H and E) (400x)

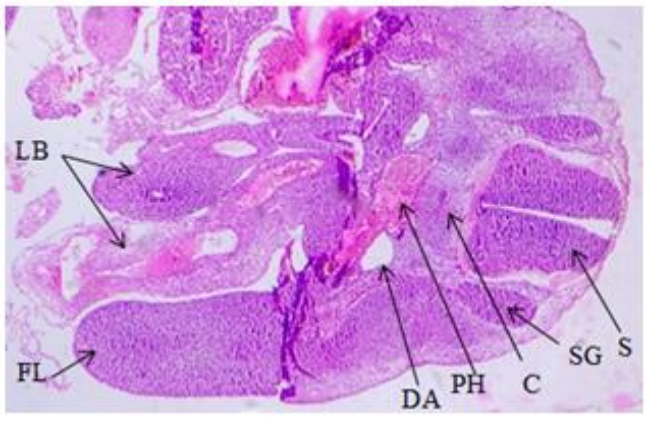

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Figure 6: Transverse section show embryos at embryonic day 11 (0.005 mg /kg Dex) (LB) Lung buds (S)Spin (SG) Spinal ganglion (DA) Dorsal aorta (PH) Pharynx (C) Chorda (FL) Fore limb (HandE) (40x).

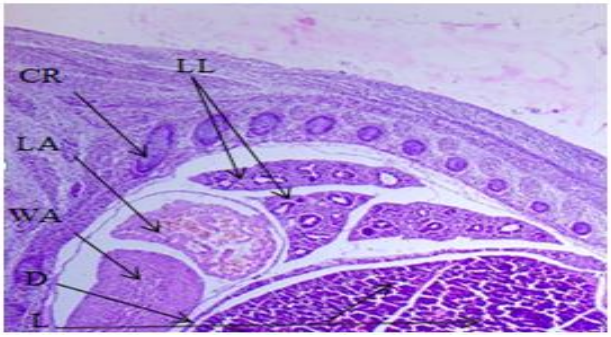

Figure 9: Longitudinal section to embryos at embryonic day 13: (WA) Wall of right vertical atrium of heart (LA) Lumen of right atrium of heart (CR) cartilage primordiumof right ribs (LL)Lobes of the lung (D) Diaphragm(L)lobes of the liver (WA) Wall of the ventricle(H and E) (40x).

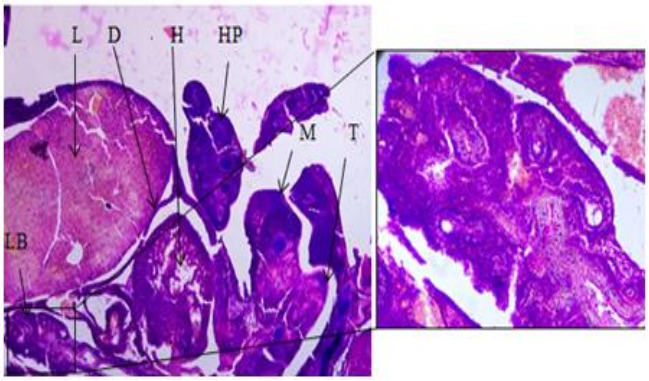

Figure7:Longitudinal section to embryos at embryonic day 11 (0.1 mg /kg dexamethasone) (L) Liver (D) Diaphragm (H) Heart (HP) Hand pave (M)Mandibular (T) Tongue (LB) Lung bud (Hand E) (40x)magnificent section to the lung bud (Hand E)(200x).

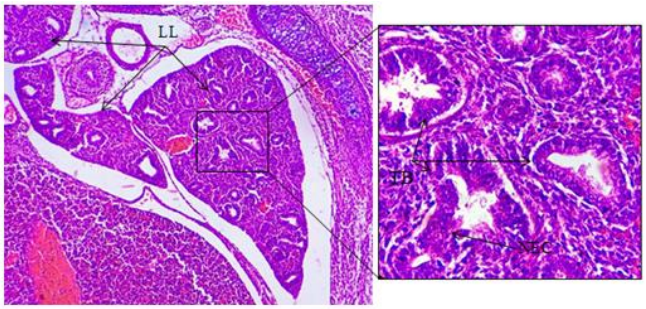

Figure 10: transvers section to embryos at embryonic day 13treated with (0.05gm/kg dexamethasone) (LL) Lobe of the lung (H and E) (100x). magnificent show terminal branching morphogenesis in (TB) terminal bud in terminal bronchioles (NEC) Necrosis (H and E) (400x).

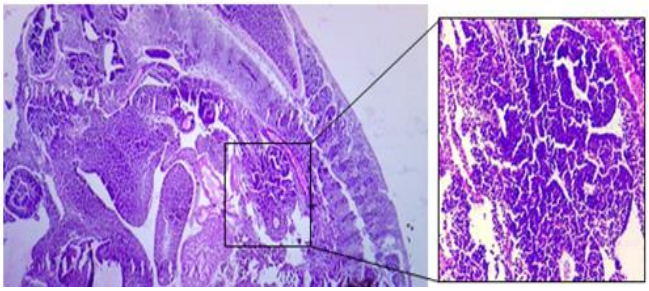

Figure 8: Longitudinal section to embryos at embryonic day 11 (0.2 mg /kg dexamethasone (Hand E) (100x).magnificent section show flower appearance to distal lung bud (H and E) (200x).

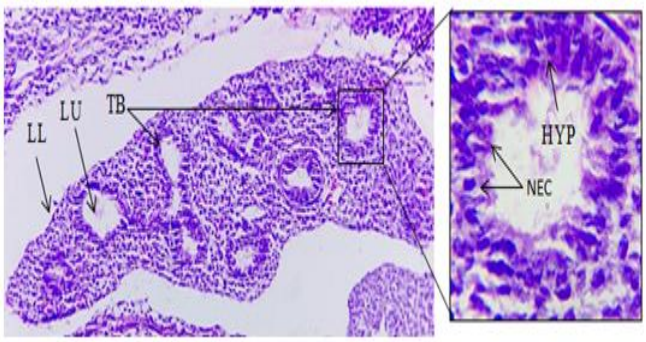

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Figure11: transvers section to embryos at embryonic day 13treated with (0.2gm/kg dexamethasone) (LL) Lobe of the lung (LU) Large lumen (H and E) (200x). magnificent show terminal bud (TB) (HYP) Hyperplasia (NEC)Necrosis (H and E) (400x).

At Embryonic day 15(Fig12) the advancing in branching morphogenesis process , there is an extensive branching of the distal epithelium and mesenchyme and the lung enter at pre canalicular stage resulting in formation of terminal sacs lined with epithelial cells(Fig 13) (Costa *etal*., 2001). Mucus hypersecretion (Fig14) in the lung at E15 may be belong to the effect of (Dex) on secretion cells and led to explosion it and secreted the mucus.

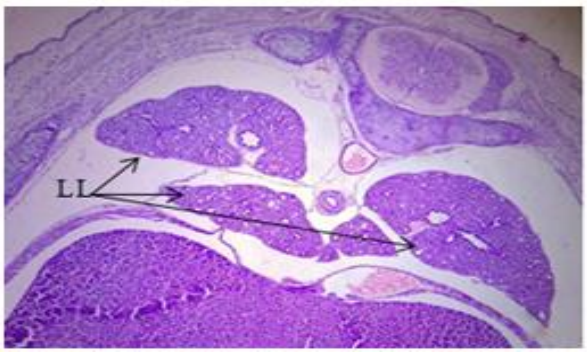

Figure12: Transvers section to embryos at E15. (LL) Lobes of the lung (H and E) (100x).

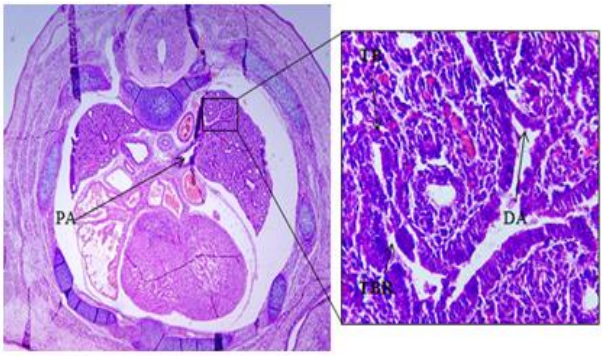

Figure 13: Transvers section through lung of embryo at E15 (0.1mg / kg Dex) (PA) Proximal airway (Hand E) (40x).and magnificent to airway show (TB) Terminal bud) (cuboidal epithelium) (TBR) Terminal bronchiole (columnar epithelium) (DA) distal airway (400x).

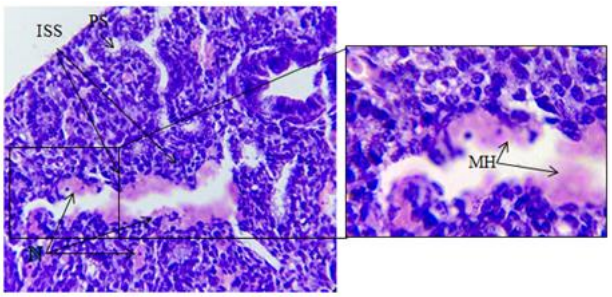

Figure14: show transvers section through lung lobe of embryo at E15 (0.2mg /kg Dex) (ISS) Intrasaccular septa (PS)Primary-saccule (N) Necrosis (Hand E)(200x). magnificent section show saccules, (MH) mucus hypersecretion (pink color) (Hand E)(400x).

3-The effect of different doses from dexamethasone on the level of alpha fetoprotein (AFP) .

AFP expressed by the embryos is transferred to the maternal blood circulation (Gabant *etal*.,2002). AFP usually indicates fetal distress or malformations, the low AFP levels can indicate growth retardation and fetal death (Mizejewsi, 1990). The increase in levels of AFP in serum during pregnancy was observed in the NTD and spina bifida while decreased level in the Dawn's syndrome (Terentive, 2013). In the current study we found different AFP concentration values as (Table 3) explains.

Table 3: Observe the different AFP concentration (ng/mg) in pregnant mice sera at different doses and embryonic days . ↑: high levels ↓:low levels

Dexamethasone mg \kg	Embryonic day		
	E 11	E 13	E15
0.00 (control group)	0.17	0.046	0.16
0.05	0.05 ↓	0.1 ↑	0.024 ↑
0.1	0.035 ↑	0.074 ↑	0.008 ↓
0.2	0.034 ↑	0.15 ↓	0.027 ↑

AFP concentration very low ,hemorrhage in the liver ,NTD , AFP synthesis in liver and yolk sac in embryonic period , the synthesis of AFP through embryonic development and its high levels in fetal serum suggest its capability to promote tissue growth (Terentive,2013) this agree with our result .

AFP elevations in maternal serum is valuable diagnostically in the detection of fetal abnormalities, particularly neural-tube defects (Thomas andTomasi,1997).

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