

# Study induce Ectopic pregnancy by diaminobenzine in Rabbits

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## Abstract

Five adult female rabbits ,their body weight is between 1.5-2 kg and their age ranges between 12-14 weeks along with 3 adult male for an probation .The animal housed in hygienic conditions at arrival immediately check for pregnancy were found negative (non pregnant). After that rod were inserted in vagina to induce puncture any part in uterus or uterine horn to administer 3,3' Diaminobenziden .After 29 days agent test for pregnancy there were found pregnant .Blood samples examined for hormonal and enzyme changes and for counting of red blood cell (RBC) white blood cell (WBC) packed cell volume(PCV) and ham globulin (Hb).There were a significant changes found in the blood values profess values and on vial the values of progesterone.

## Introduction

Ectopic pregnancy denotes a pregnancy occurring elsewhere than in the cavity of the uterus. This pathology has been recognised for years and it causes numerous maternal deaths during the first trimester of pregnancy. While this condition is well-known in humans, it is rarely diagnosed in animals(1). However, the causes and mechanisms leading to an ectopic implantation of the ovum are not always clearly defined in humans or animals. Two types of ectopic pregnancy are mainly recognized: (1) tubal pregnancy occurs when an oocyte is fertilized and then remains in the oviduct and (2) abdominal pregnancy occurs when the gestation develops in the peritoneal cavity. The latter may be subdivided into two subtypes: the primary form, when a fertilized oocyte enters the peritoneal cavity and becomes attached to the mesentery or abdominal viscera, and the secondary form, which follows the rupture of an oviduct or the uterus after the fetus has been implanted, and the fetus is expelled into the peritoneal cavity. Corneal, ovarian and cervical ectopic locations are less frequent. Several differences exist in ectopic pregnancies between human beings and animal species. While abdominal pregnancy has been described in both human and animal species, tubal ectopic pregnancies would appear to be restricted to primates. Other

than anecdotal cases (1), it is still rarely diagnosed in animals. No detailed epidemiological studies on ectopic pregnancy have been conducted on animals. In laboratory animals, a low incidence of abdominal pregnancies (0.05%) was found in pigs(2). However,(3) described a high abdominal pregnancy incidence rate in a relatively small hamster colony. The incidence is variable in commercially produced rabbits on different farms. A one-year study on the main causes of rabbit doe discard was performed on two farms where incidence rates of 7.8% and 1.6% respectively were reached for abdominal pregnancies (4).Ectopic pregnancies have also been described in other non-primate laboratory animals such as guinea pigs (5, 2), rabbits (1,6,7, 8), hamsters (3,9), rats (10, 11), and mice (12,13). Abdominal pregnancies were diagnosed in all cases except for a rat (11) and a mouse (13), where ovarian and tubal pregnancies respectively were proposed. Primary and secondary ectopic pregnancies have been proposed in all reported species, although there was no clear diagnosis at times. Although the estimation of the time the ectopic pregnancy was indicated, it was always approximate and it oscillated between a period of days to 3 months in a rabbit with a primary abdominal pregnancy (1).

## Material and method

Five adult female rabbits at the age of 1.5-2 kg BW and Then age ranging between 12-14 weeks along with three adult male for from natural service, brought the local market of Basra .All the animals were kept under completely hygienic condition and rain on a stander feed.The chemical agent used to induce octopic pregnancy as 3,3' Diaminobenziden Hydrochloride ,3,3-4,4 bibphenyltetramine tetrahydrochloride (DAB),at dose rate 0.5 mg/kg (iu).Before injecting DAB powder dissolved in corn oil and store in aliquots at temperature degree at 20c° for farther use. It should not be kept in freeze.

### The method

On next day of animals arrival a pregnancy test were done to all the female to find out if there is any pregnancy and it comforted that the animal were not pregnant .The rod were inserted per vagina to induce puncture any part in uterus or uterus horn to simultaneously the administer there 3,3 'diaminobenziden (DAB) were done .At this time the animal left for natural service 29 day after a pregnancy test were done ,It is was positive .

Here 4ml blood draconic with the help of sterile disposable syringe (5ml) directly from the heart.The drown blood divided into to equal part that is 2ml each .One part used anti-coagulant (EDTA)for hematological studies while the used for serum preparation for biochemical analysis .

### Red blood cells (RBC) (Cell/mm<sup>3</sup>)

The RBC count was done by the use of haemocytometer (improved Neubauer double) and (Hayme's solution) and special pipette for dilution.The blood was sucked by specified pipette to mark 0.5 the haemocytometer RBC count, then was diluted by (Hayme's solution)by sucking to the mark 101, and the pipette is stirred horizontally to mix the solution, then some of the liquid drops are split to elevate the non diluted solution, after that the special slide of the appliance is filled and covered then left for few minutes to permit the

cells to settle over the counting squares area. The cells are counted in five middle sequares of 25 sequares (4 sequares in the 4 angles and one sequare in the middle) by the use of high microscopic amplification, then the following equilibrium of corpuscles counting (Cell/mm<sup>3</sup>) was No RBCs = No. × 10000

### Hemoglobin concentration (Hb) (g/dl)

The concentration of Hb was measured by the use of (Sahli appliance). where 10 ml of HCl of 0.1 normally is poured to Sahli tube and 10µl of blood specimen is add and mixed together, then the mixture is let for 10 minutes, and later on distilled water is add gradually till the colour of the blood tenders similarly to that of the standard tube colour.Packed cell volume (PCV) (%).The microhematocrit method was used to calculate the percentage of PCV by the use of capillary tubes which contain heparin, where one end of which was closed by artificial clay after being filled to 3/4 of its length with blood, and put in microcenterfuge on velocity 1200 rotation/minute for five.minutes, then the hematocrit value was obtain by Service device (14).Total White blood cell count (WBC) (Cell/mm<sup>3</sup>).The WBC was obtained by the use of haemocytometer (Neubauer improved double) and (Turk's solution) and special pipette for dilution (15).To obtain WBC count Turk's solution was used for cells dilution, where the blood is sucked to the mark 0.5 by the pipit then its filled to the mark 11 by the diluting solution, then the pipette is stirred horizontally to get blood mixed, the non diluted solution is elevated by spilling some drops, then the glass slide is filled and covered and left for few minutes to permit the cells to settle over their squares, The cells are counted in four large squares found outside the four angles of the large square used for counting RBC, then the following equilibrium is applied to the total WBC count (Cell/mm<sup>3</sup>):Total Leukocyte counts = No × 50

### Differential WBC count

After the blood smear was done, the slide was stained with leishman's stain for 10 minutes then it was washed with water to eliminate the over stain, and left to dry,

then examined under oil immersion power to count the percentage of each type of WBC (14).

### **Hormonal Assay**

Progesterone concentration measurement in human: Use progesterone enzyme immunoassay test kit catalog number: BC-1113. From bio check, Inc 323 vintage park Dr. Foster city, CA94404

#### **Principle of the test:**

The bio check progesterone EIA is based on the principle of competitive binding between progesterone in the test specimen and progesterone-HRP conjugate for a constant amount of rabbit anti progesterone.

#### **Procedure:**

In the incubation, goat anti-rabbit IgG-coated wells are incubated with 25 MI progesterone standard, controls, patient samples, 100 MI progesterone-HRP conjugate reagent and 50 MI rabbit anti progesterone reagent at room temperature 18-25C° for 90 minutes. During the incubation, fixed amount of HRP-labeled progesterone competes with the endogenous progesterone in the standard, sample or quality control for a fixed number of binding sites of the specific progesterone antibody. Thus, the amount of progesterone peroxidase conjugate immunologically bound to the well progressively decrease as the concentration of progesterone in the specimen increases. Un bound progesterone peroxidase conjugate is then removed and wells washed. Next a solution of TMB reagent is then added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured at 450 nm wave length on Elisa reader (Spectrophotometrically). Estrogen (E2) concentration in human serum: Used estrogen enzyme immunoassay test kit catalog number: BC-1111. From bio check, Inc 323 vit age park Dr Foster city, CA 94404

#### **Principle of the test:**

The bio check (E2) EIA is based on the principle of competitive binding

between E2 in the test specimen and E2-HRP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti rabbit IgG-coated wells are incubated with 25 MI E2 standard controls patient sample 100 MI estradiol-HRP conjugate reagent and 50 MI rabbit anti-estradiol reagent at room temperature (18-25 C°) for 90 minutes. During the incubation, a fixed amount of HRP-labeled E2 competes with the endogenous E2 in the standard, sample or quality control serum for a fixed number of binding sites of the specific E2 antibody. Thus the amount of E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Un bound E2 peroxidase conjugate is then removed and the well washed. Then, a solution of TMB reagent is added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1 NHCL and absorbance is measured by Eliza reader at 450nm wave length.

#### **Histological technique in females rabbit:**

Preparation of tissue and Routine Hematoxylin-Eosin staining method. Then after the animal sacrificed to observe the condition of uterus and fallopian tube, and local the file pregnancy .specimen from uterus ,placenta ,liver was take for histological examination .

The specimen fixed in buffered formalin 10%. Dehydration was done by passing specimens in increasing concentration of ethanol ,infiltrated 3 times with xylene or chloroform and embedded in paraffin. Five -micron thick sections of paraffin embedded tissue specimens were mounted on glass slide and stained with hematoxylin -eosin. Sections were examined by mean of light microscope study according to (16).

#### **Statistical analysis:**

Results are expressed as mean± Standard deviation (SD); means were compared by two-way analysis of variance ANOVA, one-way ANOVA, and T test and least significant difference

(LSD) for comparison. Difference between mean data were considered significant at

( $P < 0.05$ ) according (SPSS).(17).

### Result and Discussion

The changes in blood parameter was shown in table (1) and (2) where all the values of RBC, PCV and Hb are significantly very height comparing that of control value. The value of RBC  $\times 10^3$  animal treated with diaminobenziden is  $8.2 \pm 0.68$  while in control animal is  $3.05 \pm 0.039$  similarly value of PCV in treated animal is  $30.00 \pm 1.64$  while that of control is  $15.0 \pm 0.27$  and the of Hb gm/dl is  $10.6 \pm 0.43$  in treated animal while that control is  $5.45 \pm 0.071$ . Table (2) shows the significant increase in the percentage of differential leukocytes. The total WBC  $\times 10^3$  cell/mm<sup>3</sup> in diaminobenziden treated animal  $6.48 \pm 0.02$  and that 9 control just a half of the value that is  $3.24 \pm 0.01$ . Nutrophile, lymphocyte, monocytes in DAB treated animal  $60.2 \pm 2.75$ ,  $35.3 \pm 1.92$  and  $10 \pm 0.52$  respectively. However the of Eosin in DAB treated animal is significantly decreased from that 9 control

group while the DAB treated animal is  $1.5 \pm 0.05$  while that of control is  $2.16 \pm 0.35$ . However table (3) shows significant decrease in the value of progesterone and estrogen. In control group the progesterone were  $1.21 \pm 0.16$  and estrogen  $53.55 \pm 11.825$  while that of DAB treated animal is  $0.14 \pm 0.02$  progesterone and  $1.3 \pm 12.5$  estrogen that because the pregnancy doesn't take place in the uterus but in abdominal cavity for the reason the total leukocyte increased to double value (4). In table 4 were total protein in DAB treated animal significantly decreased than of control group. The same result shown on Bilirubin. Histological finding is shown through figure No1. Slide taken from placenta show area of muscular necrosis and calcification. This is similar to the finding reported by (18). figure 2 show higher magnification.

Table(1): Effect 3,3' Diaminobenziden on total RBCs count, P.C.V and hemoglobin concentration (Hb conc.) in rabbits. (means  $\pm$  SD.).

Parameter Groups	RBCs $\times 10^3$ cells/mm <sup>3</sup>	P.C.V %	Hb Gm/dl
Control N=5	$3.05 \pm 0.039$	$15.0 \pm 0.27$	$5.45 \pm$
3,3' Diaminobenziden N=5	$8.2 \pm 0.68$	$30.00 \pm 1.64$	$10.6 \pm 0.43$

Table(2) Effect 3,3' Diaminobenziden on total WBCs count and differential in rabbits. (means  $\pm$  SD.)

Parameter Groups	WBCs $\times 10^3$ Cell/mm <sup>3</sup>	Nutro %	Lympho %	Mono %	Eosin %
Control N=5	$3.24 \pm 0.01$	$16.5 \pm 0.96$	$26.83 \pm 0.55$	$4.15 \pm 0.21$	$2.16 \pm 0.35$
3,3' Diaminobenziden N=5	$6.48 \pm 0.02$	$60.2 \pm 2.75$	$35.3 \pm 1.92$	$10 \pm 0.52$	$1.5 \pm 0.05$

Table(3)Effect 3,3'Diaminobenziden on Hormone in rabbits. means± SD.)

Parameter Groups	Progesterone ng/ml	Estrogen Pg/ml
Control N=5	1.21 ± 0.16	53.55 ±11.825
3,3' Diaminobenzide N=5	0.14 ± 0.02	1.3± 12.5

Table (4) Effect 3,3' Diaminobenziden on total protein in rabbits .(means± SD.)

Parameter Groups	Total Protein g/dl	Bilirubin Pg/ml
Control N=5	0.194 ± 0.24	0.128 ±0..04
3,3' Diaminobenziden N=5	0.097±0.012	0.064±0.02

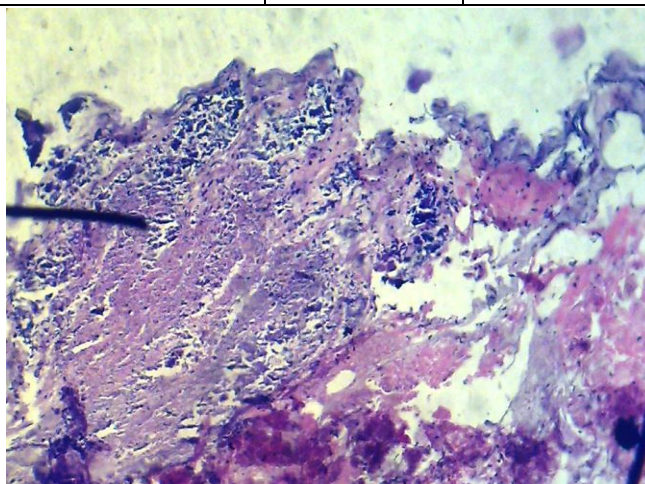


Fig.(1) Placenta. areas of Muscular necrosis and calcification.

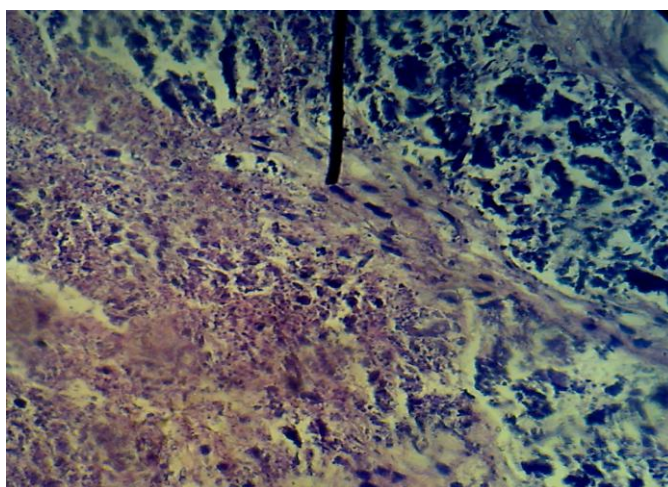


Fig.(2) Placenta. Higher magnification, areas of necrosis with Calcification

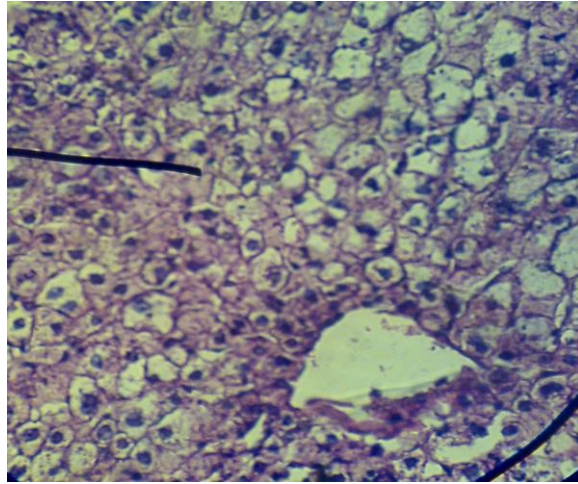


Fig.(3)Liver.Not/Moderate vacuolation of hepatocytca

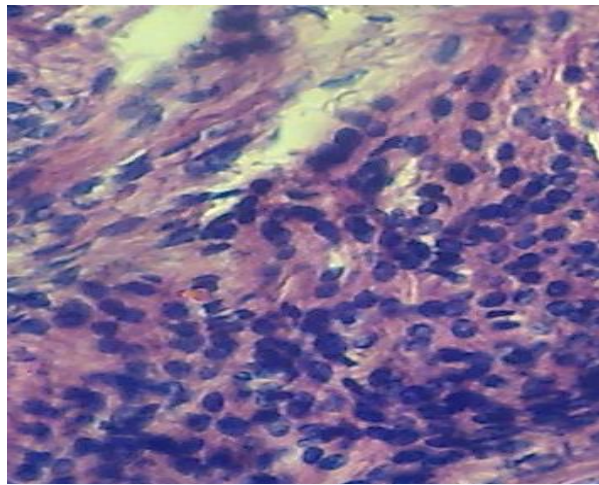


Fig.(4)Uterus. Mixed inflammatory cells as neutrophils, mononu oclal cells.



Fig.(5)Rabbit doe.Eight different sized fetuses (black asterisks) (A) and floating free in the abdominal cavity.





Fig.(6) Rabbit doe.Recent abdominal pregnancy secondary to a left horn rupture (arrow).Two fetuses showed placental attachments to different abdominal surfaces.

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### الخلاصة

اجريت التجربة على خمسة أرناب إناث بالغة بوزن يتراوح ٥-١, ٢-١ كليو غرام وبأعمار يتراوح بين ١٤-١٢ أسبوع سوية مع ٣ ذكور بالغين تحت التجربة والحيوانات في الشرط الصحي من أجل فحص الحمل فوراً وكانت النتيجة سالبة (غير حبلية) بعد ذلك تم إدخال القضييب بواسطة المهبل لحدوث نقب في أي جزء في الرحم أو قرن الرحم بواسطة 3,3'diaminodenziden وبعد ٢٩ يوم من اختبار الحمل كانت هناك في عينات الدم وجود حمل وايضاً يلاحظ تغيرات هرمونية وإنزيمية وتغيرات بحساب خلية الدم الحمراء وخلية الدم البيضاء وكريات الدم المرصوصة وهيموغلوبين وكان هناك تغير معياري في قيم الدم في القارورة يتم تسجيل البروجسترون .