ABSTRACT

The effects of electromagnetic fields (EMFs) with a maximum 138 gauss and 100 Hz frequency was applied on the development of Balb/c mouse strain embryos in this work. Pregnant mice aged 2.5 - 3 months old after vaginal plug observation were exposed to EMFs once for 10 hours in days 5, 6 and 7 of gestation separately.

Simultaneously groups of sham - exposed and controls were used in each experiment.

In all experiments the embryos were extruded in day 15 of gestation, studied morphologically and histologically. The experimental embryos of day 5 gestation showed a significant increase in CR measurements ,body and placenta weights ($p < 0.05$) and abnormal development of vertebral column.

In %17.1 of the cases the embroys with their placentas were atrophied, also %30.4 of placentas in maternal surface contained increased numbers of Hofbauer macrophage cells.

The experimental embryos of day 6th gestation showed significant increase in CR measurements ($p < 0.05$).

The embryos revealed morphological features of day 17 and 18 of gestation when extruded in day 15. These expereimental embryos had a thick and wrinkled skin, the eye lids and pinnae were formed completely, these changes were due to their accelerated grwoth and development.

The gestation period decreased in this series of experiments and in some casses the litters were born in day 17 - 18 (normal days 19 - 20) of pregnancy.

The experimental embryos of day 7 of gestation showed %9.4 abnormal curvature in head - tail axis and neural tube.

CR measurement and their body weights had significant increase ($p<0.05$) in these series of experiments.

Abbreviations:

EMFs : Electromagnectic fields
CS : Cross section
Sg : Sagital section
Ft : Frontal section
CR : Crwon rump measurements

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Introduction

In recent years the effects of EMFs on biological material have attracted many workers. Goodman, et. al (1979) studied the effects of 0.1-2 gauss intensity on Physarum polycephalum and found changes in respirations and cell divisions. Edelman et. al (1974) reported the effect of magnetic fields on frog sciatic nerve. Basset (1981, 1982) gained some success in bone fracture repair using pulsing EMFs. Lubin, et. al (1982) studied EMFs stimulations on bone and bone cell in vitro.

There are various contradictroy results from the effects of EMFs on development of different embryos. Studies made by Delgado, et. al (1982) using 10,000 and 1000 Hz EMFs and Juutilainen et.al (1986 a,b) on chick embryos resulted considerable defects and Maffeo (1984, 1988) using 120 m gauss EMFs did not find significant embryonic malformation in chick. Studies of Nishikava (1987) using 5-19 gauss EMFs during prenatal and postnatal developmental stages also did not show remarkable, malformations in mice.

In 1988 Heinricks by application of 0.35 Tesla (1 Tesla = 10^4 gauss) EMFs in day 9 of gestation for 16 hrs reported CR decrease in 18 days Balb/C mouse embryos. Mcgivern (1990) worked out the effct of 8 gauss EMFs on rat embryos and reported weight increase of sex organs and in his studies effects of EMFs on the litters did not change the levels of testosterone, FSH and LH till day 120. Zusman (1990) used 20 and 50 Hz EMFs in mouse and rat embryos reported %50 undeveloped blastocysts, telencephalon and optic vesicle, also decrease in CR measurements. Tyndall (using a terapeutic magnetic resonance im (MRI) dose of 1.5 Tesla in day 7 of ges found eye maldevelopment in C57 Bl/6I r embryos. Also there are reports indicating EMFs produced from high voltage power increases leukemia in resident human indiv around them (Wertheirmer and Leeper 197 Wertheimer, 1983).

Material and Methods

Balb/C mice strain obtained from Institue of Pasteur, Tehran were used as a model in this investigation.

The EMFs coil was constructed departmental workshop. The coil consissted plastic cylinder 24.7 cm length, 13.04 cm diameter and around it 6 layers enamelled co wire 0.95 mm in diameter with 250 turns in layer were applied. To uniform the EMFs bet each layer of the coil a sheet of solenoid was The magnetic field strength at the center o coil was calculated from the follwoing formul

$$B_m(\text{gauss}) = \mu o \; GIm \sqrt{\frac{Rf}{\rho ro}}$$

Where:

B_m = maximum magnetic field

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μo = permeability of vaccum = 1 uemcgs

G = constant which depends on geometrical property of coil and current distribution.

I_m = maximum current = 2.13 A

R = resistance of coil (Ω)

$$f = \frac{\text{volume of the wiring}}{\text{total volume of the coil}} = \frac{Vc}{Vt}$$

ρ = Resistivity of copper = $1.8\ 10^{-6}$ Ωcm

ro = inner radius of the coil (cm)

G is calculated from:

$$G = 0.2 \sqrt{\frac{2\pi\lambda}{\alpha^2-1}} \times \ln \left[\frac{\alpha+\sqrt{\lambda^2+\alpha^2}}{1+\sqrt{\lambda^2+1}} \right]$$

Where :

$$\lambda = \frac{l}{2ro}, \alpha = \frac{r}{ro}$$

l = lenght of coil (cm)

r = outer radius of coil (cm)

V_c = LA

L= total length of wire = 592 m

A= cross section of wire = $7.08\ 10^{-3}$ cm^2

$V_t = \pi l (r^2 - r_o^2)$

Specifications of coil:

r_o = 6.25 cm

r = 7 cm

l = 24.7 cm

R = 15 Ω

The function generator used in this work was full wave rectifier. Using such and equipment and design of EMFs we could obtain a field with maximum 138 gauss and 100 Hz (Fig 1). In each experiment in a small plastic cage. (8 cm lengnth, 3 cm width and 4 cm hight) in days of 5, 6 and 7 of gestation were placed in the center of EMFs for 10 hours. In each series of experiment sham, exposed and controls were used. In day 15 of gestation the pregnant mothers were killed and their embryos extruded. The embryos were whashed with saline solution, weight and CR measurements was applied separately. After morphological observations the embryos were fixed in Bouin's fluid for histological investigations. Serial cs, ft and sg sections (6 μm thickness) were prepared and the slides stained with H & E. The whole mount embryos photographed with Zeiss stereophotomicroscope model DR and the sections with Zeiss photomicroscope model M_2.

Analysis of variance was applied as statistical methold.

In another series of experiments 5 pregnant mothers were exposed to EMF and allowed to approach the term. These experiments were set up to compare normal and experimental delivery time.

Results

The effects of EMF with 138 Gauss intensity in a frequency of 100 Hz on the development of Balb/C mouse embryos investigated. Observations on the 5 days old embryos showed increase in CR, weight and placentas measurements (fig 2). %8 of the embryos of this day of gestation had lordosis defect (Figs 3,4). In %10 of the experimental

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embryos abnormal carvature in the vertebral column were observed (Fig 5), and %20 of the embryos were atrophied. The embryos were atrophied in different stages of development (Fig 6). The placentas of experimental embryos showed different degrees of malformation, they were lagrer than sham-exposed and controls (Fig 7). In some cases they had atrophied pieces remained tighty in maternal surface (Fig 8).

Serial sections of experimental placentas showed atrophied pieces in maternal surface, tissue disorganization irregular decidual septa and increase of macrophage cells in atrophied areas (Figs 9, 10)

Experimental embryos of days 6 of gestation showed the most malformation in this work, CR measurements showed extreme increase and the weights of embryos and placentas increased considerably (Fig 11).

Despite of accelerated growth the development in 6 days embryos their eyelids were closed but the lens and retina had normal development (Figs 12, 13). The external ears showed considerable defect, the pinnae were collapsed and meatus had no external ostium (Fig 14, 15). These charcteristics are similar to the embryos of day 17-19. The embryos of these series of experiments were traced up to day of birth. The results showed decrease in the length of pregnancy.

Weight measurement of the litters showed significant increase (Fig 16). The embryos of 7 days gestation reveald modifications in body curvature towards the left side in vertebral column, (Fig 17,18). Histological observation embryos showed defects intervertebral di of the discs did not develop in normal fe their height were shorter than the condition. The weight of placentas comparing with the control individuals series of embryos (Fig 19).

Discussion

Although Conghill (1990) and som researchers introduced EMFs as killing seems there remains many works to be this line of research. It is obvious that different intensities produce various alter the molecular and cellular levels of organisms (Pool, 1990). The induction replication and RNA transcription ha worked out by Goodman et al (1983) on under EMFs influence. EMFs have effects surface (Marron et al 19983) on ju complexes (Lubin, 1982) and on intra ca^{++}concentration (Jolly, 1983). Liboff (19 reported effects of 2.3-560 μT increase synthesis. Studies on different literature rev EMFs are not mutagenic agents but th strong reasons for teratogenicity of EMFs.

Experiments carried out by Delgado Ubeda (1983), Cameron (1985), Rooze Juutilainen (1986) and others on the embryos under the EMFs in different freq each revealed specific results. Applicati EMFs with 100 Hz and 1.2 μT inten Delgado has shwon supperssive effects on

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embryo develoment this result has already been agreed with other investigators. Experiments of Zusman (1990) using low EMFs on the mammalian embryos did not show significant results in vivo conditions. We also did not get significant results in the same fields. The effects of high EMFs with 138 gauss resulted remarkable deffects in particular in day 6 of gestation. It is concluded that by using low field the mother's protective systems both in molecular and organ levels maintain the embryonic development in normal conditions but in the high fields these systems are not efficient.

Experimental embryos in our work in day 5 of gestation showed a significant increase in CR measurements, body and placenta wights ($p<0.05$) (Fig.5) and various results including lordosis, maldevelopment of vertebral column; since on this day of development the egg cylinder is formed. According to Delgado (1982) it can be postulated that, changes in orientation of glycosaminoglycans could affect the embryonic tissue development resulting various defects and atrophied embryos. Also it is resultesd that day 5 of gestation is a very high critical period to the mouse embryos in high intensity EMFs. In comparision, the embryos of day 6 of gestation showed completely different results; growth and development was remarkalbe and no atrophied embryos were observed. The experimental embryos in day 15 of gestation are comparable with day 18-19 of normal development from CR and weight measurements points of view. The skin of day 6 embryos under EMFs effects are similar to the term embryos, with a very thick, wrinkled and though appearacne. Results of day 7 of gestation is contracdictory with day 6. In these embryos no significant defect except a curved appearance in vertebral column were observed and this may be is due to the presomite stage defects of day 7. comparing all of the statistical results of this work on the CR measurements, body and placenta weights, a significant increase is observed on these parameters, Figures: 2, 11, 16 and 19 ($p<0.05$). It is conluded that high intensity EMFs can be considered as mitogenic, teratogenic and mutagenic agent.

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Fig1. Schematic drawing of the EMF system used in this work. A., ampermeter; c, coil; G, generator; Os, Osciloscope

Fig2.comparsion between CR, weights of embryos and placentas (day 5)

Fig 3. Stereophotomicrograph of control embryo in day 15 of gestation. Ey, eye; E ear; FL, forelimb; HL, hindlimb; T, tail. X 28.

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Fig 4. Stereophotomicrograph of experimenal embryo indicating lordosis defects. X 28

Fig 5. Stereophotomicrograph of experimenal embryo shwoing abnormal curvatur in the vertebral column. X 28

Fig 6. Stereophotomicrograph of cross section from the uterus, placenta and atrophied embryo X 78.5.

At, atrophied embryo; Pl, atrophied placenta; U, uterus.

Fig 7. Stereophotomicrograph of control (left) and experimental placenta with a large size (right). X 28

Fig 8. Stereophotomicrograph of control (left) and experimental placenta with a large size (right) note to the atrophied placental pieces in maternal surface, Atp. X 28

Fig 9. Photomicrograph of a section through maternal placenta with normal number of macrophages. BV, blood vessel; MP, macrophage, X 446

Fig 10. Photomicrograph of experimental placenta with large number of macrophages. Mp, macrophage. x 446

Fig 11. Comparision between CR. weights of embryos and placenta (day 6)

Fig 12. Stereophotomicrograph of section through the control embryonic he c, cornea; L, lens; Ld, eye lid; Nc, nasal c 28

Fig 13. Stereophotomicrograph of section through the experimental embryo region. (treated in day 6). Note to the ac growth of eye lids. Ld, eye lid. X 28

Fig 14. Stereophotomicrograph of section through the control embryonic hea Ee, external ear; Ie, internal ear. X 28

Fig 15. Stereophotomicrograph of section through the experimental embryo region. (treated ind day 6). Ee, externa internal ear. X 28

Fig 16. Comparision between the we neonates. (day 7)

Fig 17. Stereophotomicrograph of section trough the neural tube and v column of control embryous. Nt, neural t ribs; V, Vertebra. X 57

Fig 18. Stereophotomicrograph of section through the neural tube and v column of experimental embryos. (treated shwoing abnormal bending appearance. x 57

Fig 19. Comparision between CR; wei embryos and placentas (day 7)

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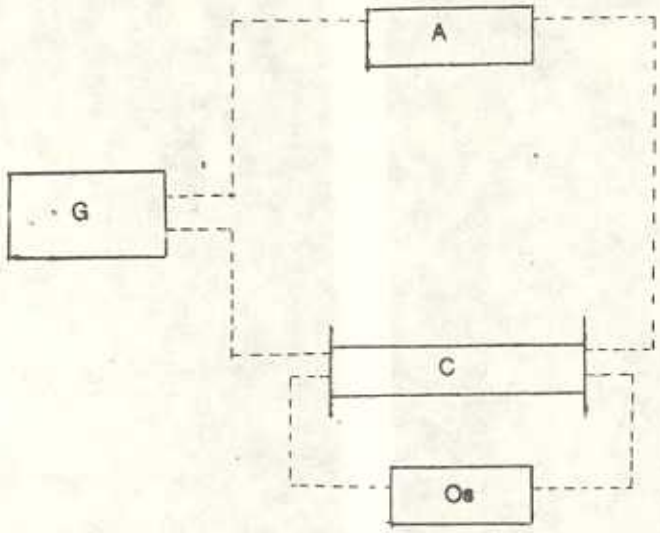
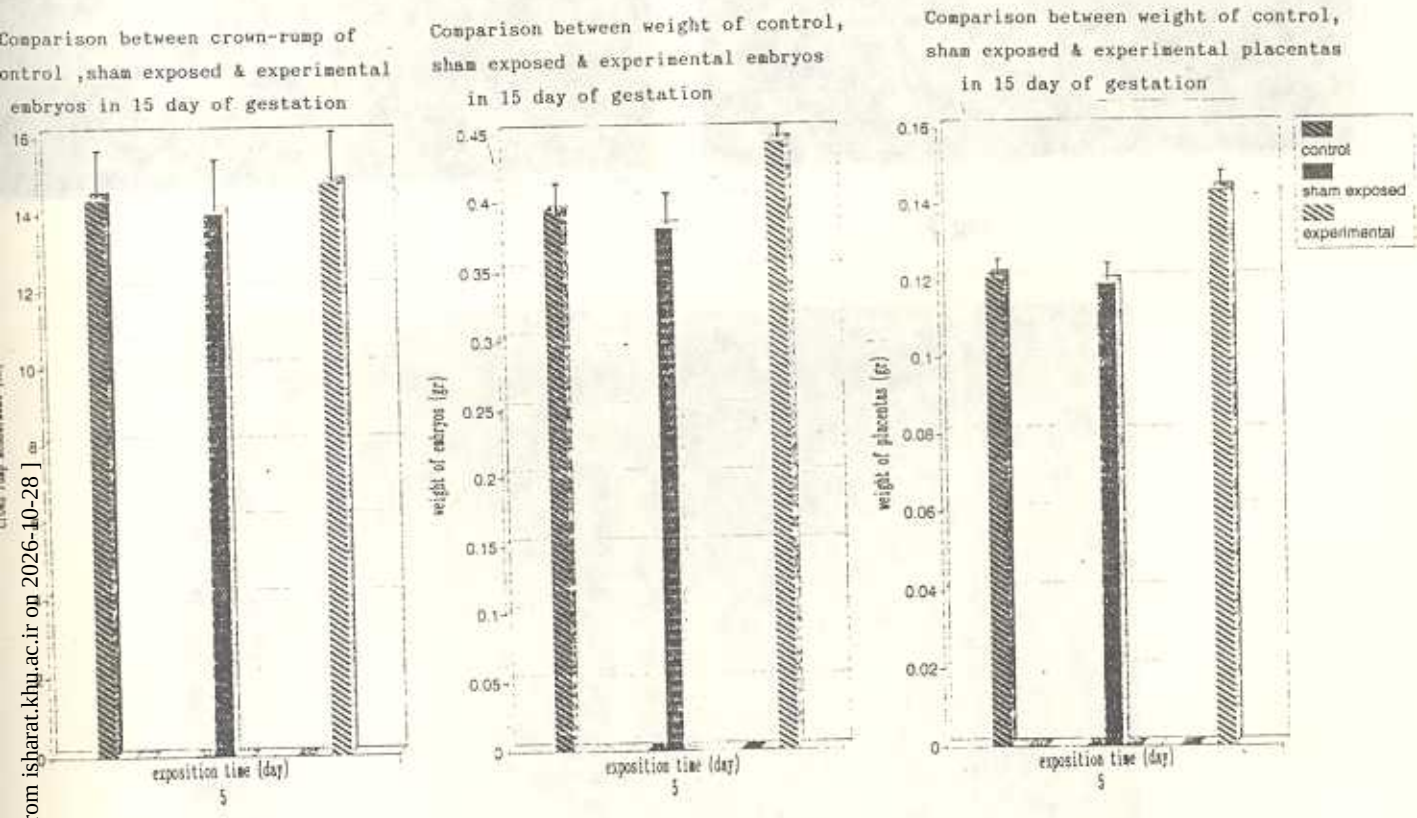


Fig·1

Fig 1



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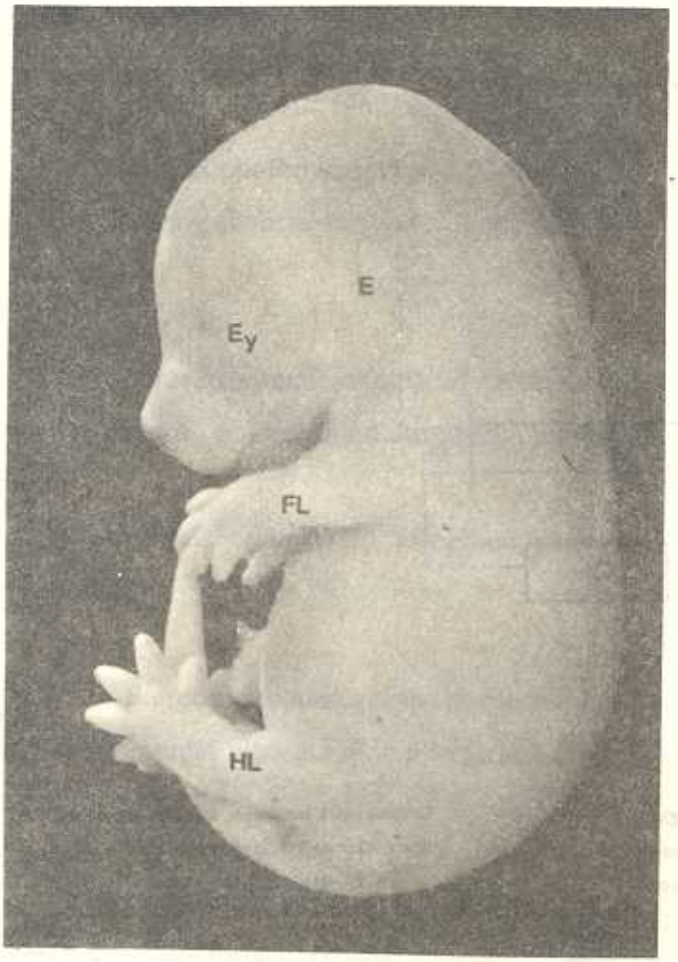

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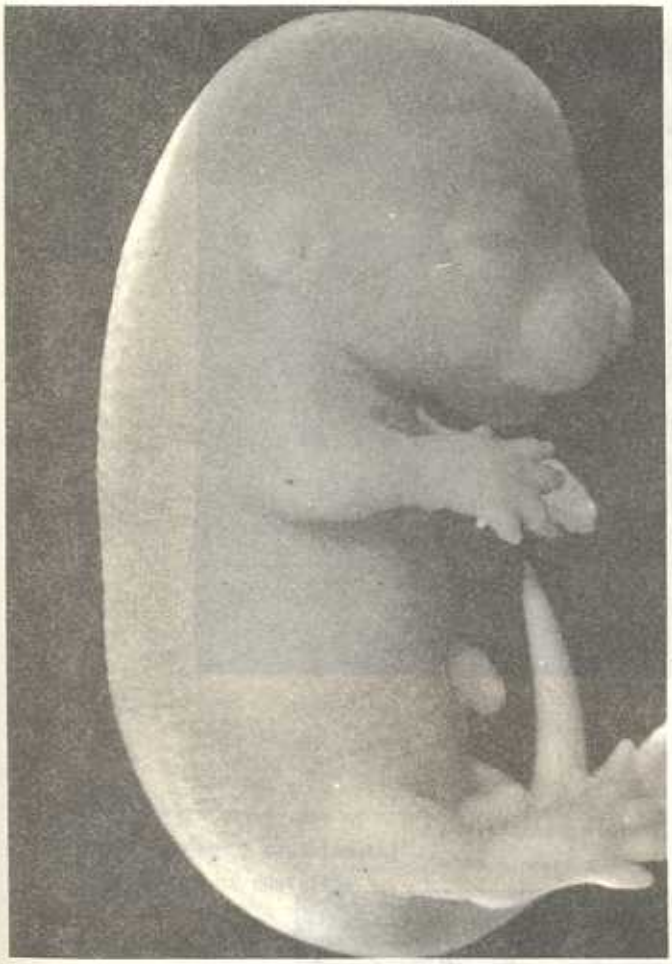

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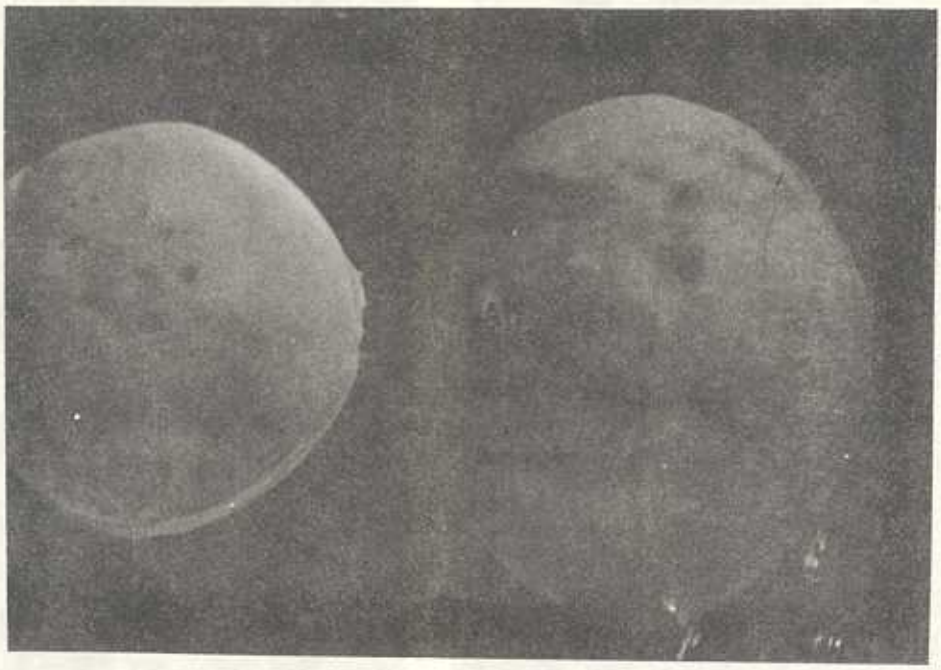

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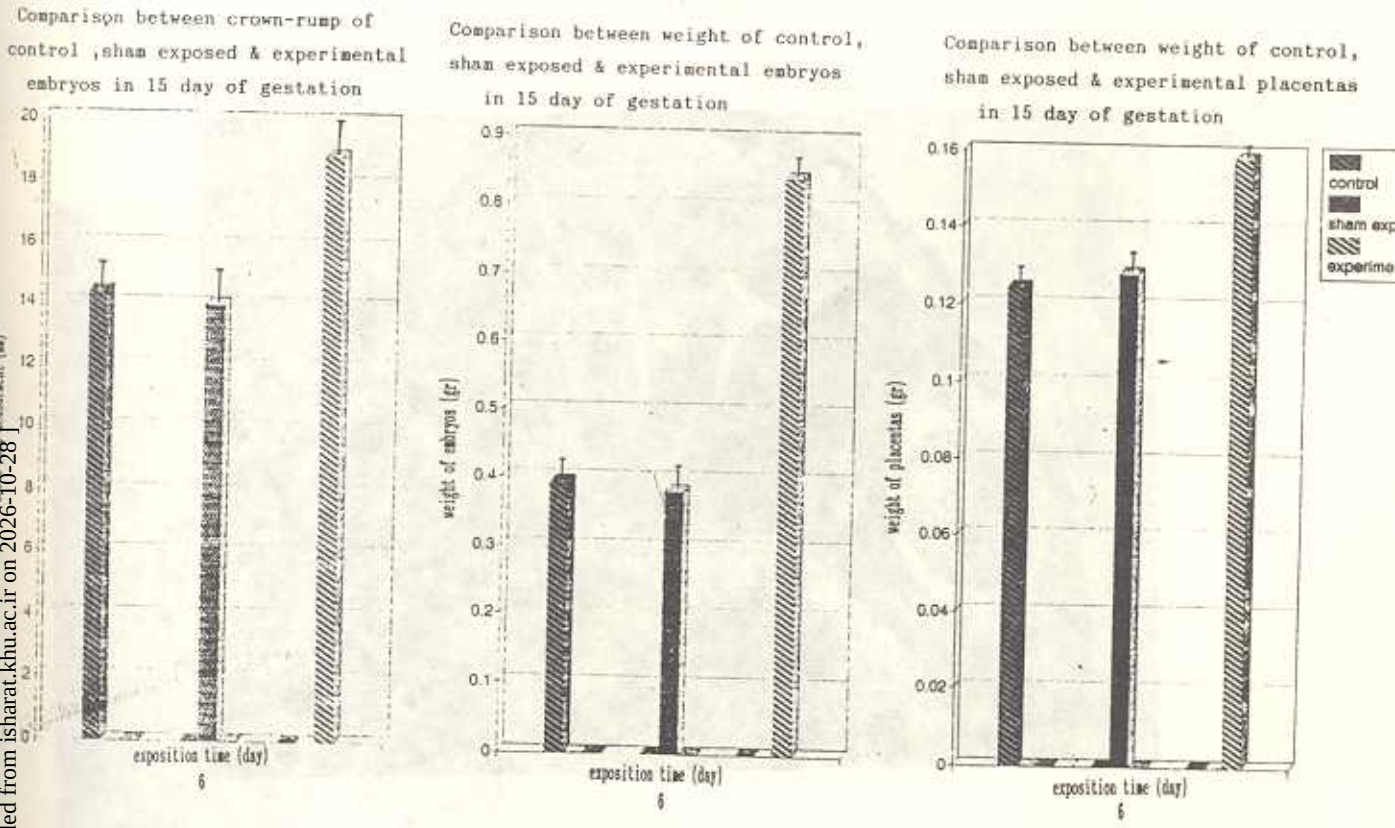

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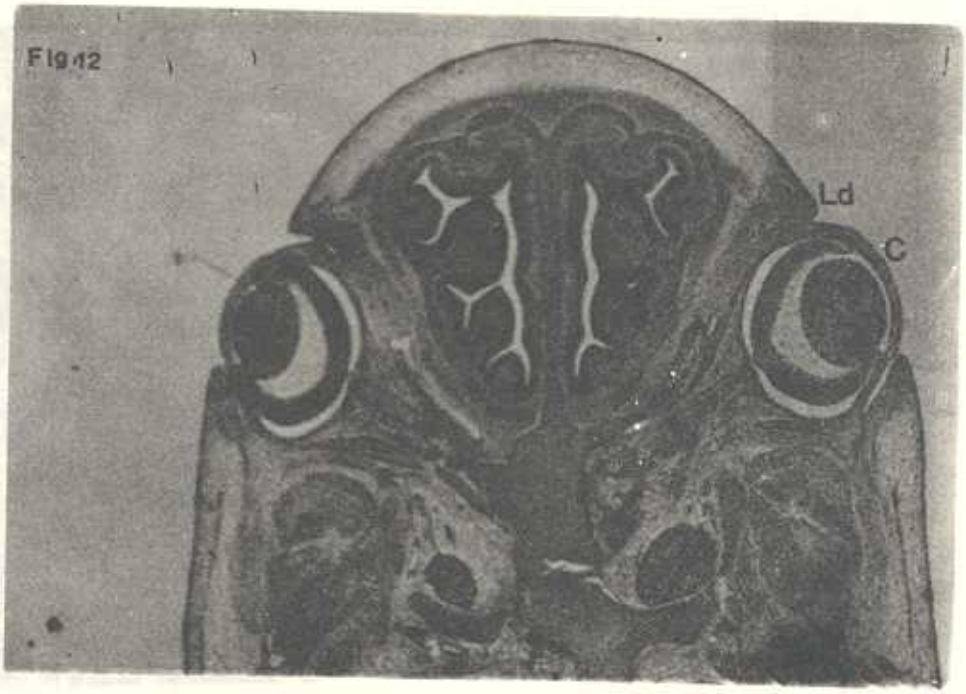

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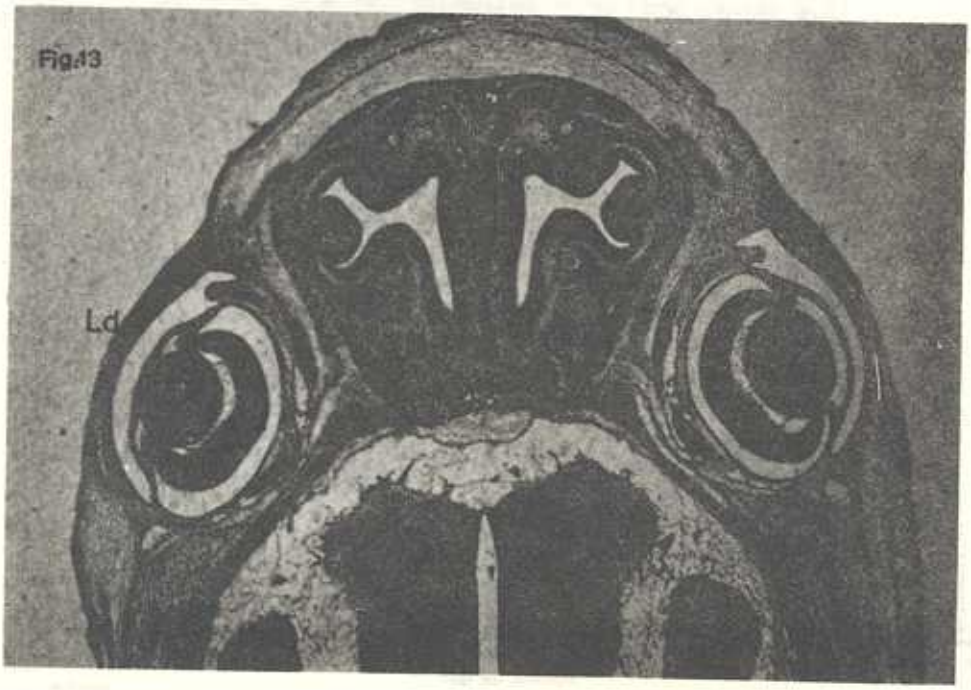

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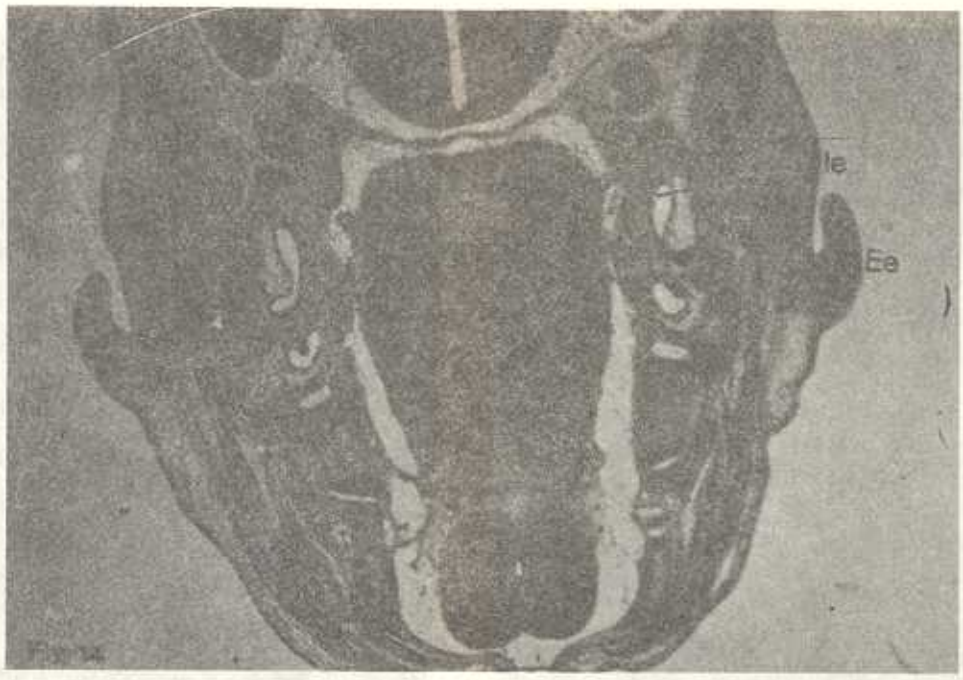

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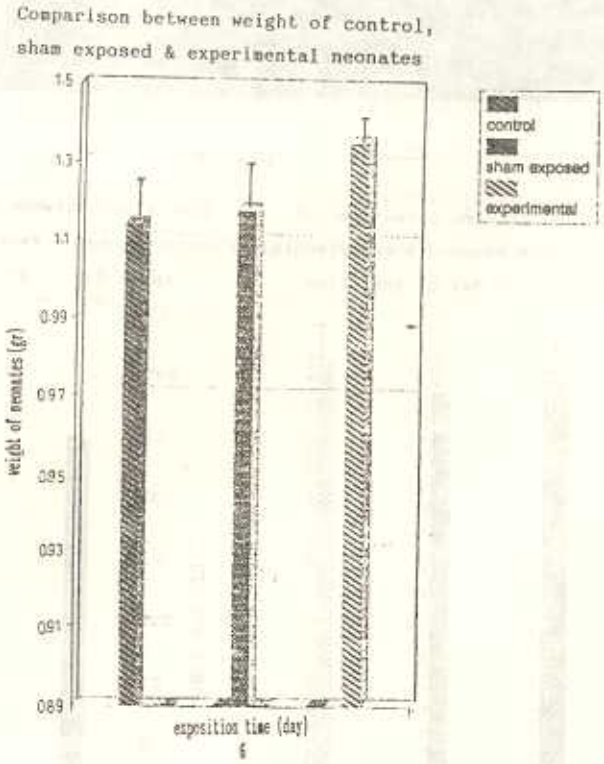

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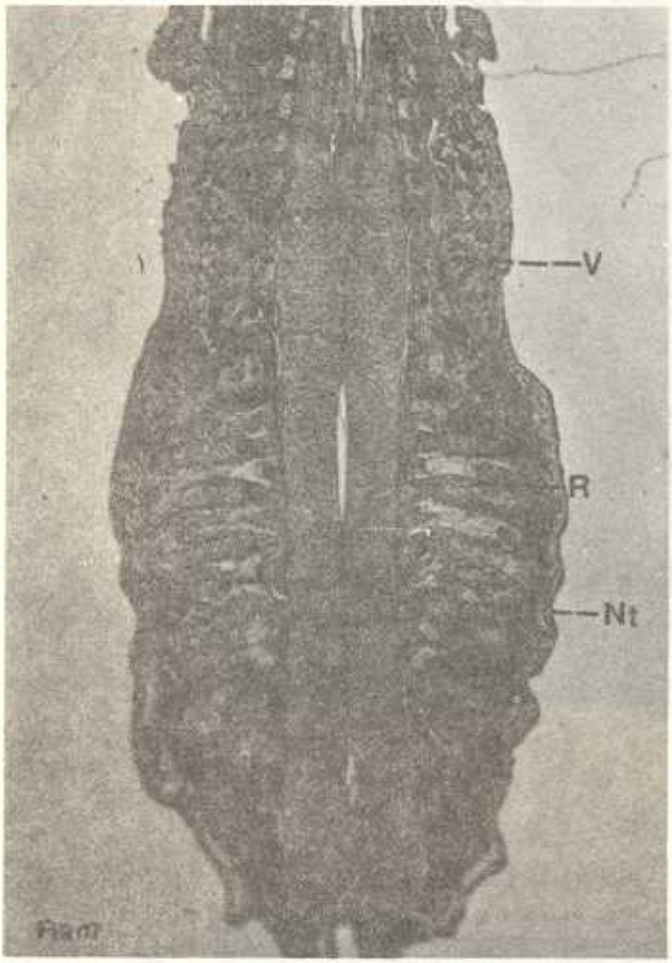

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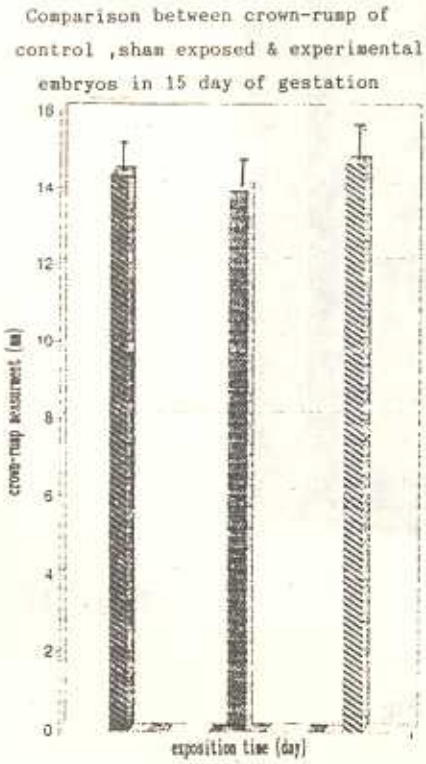

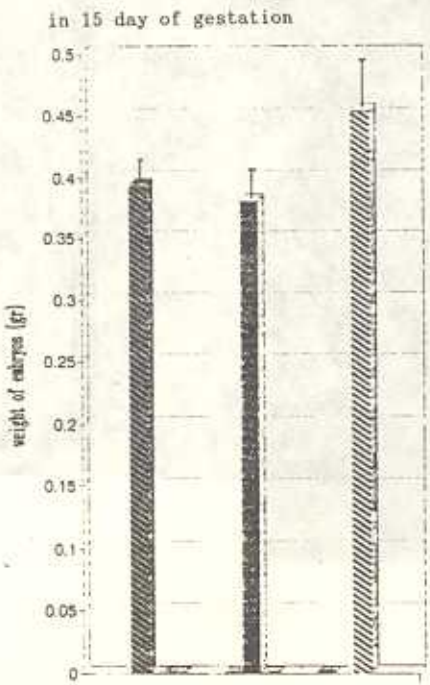

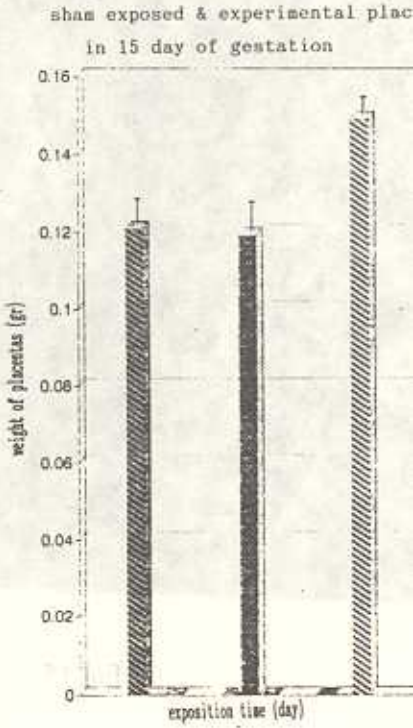

Fig 19

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