

FETAL ALCOHOL SYNDROME IN FETUS OF MOUSE

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Abstract — This study is based on embryotoxic effects of ethanol on embryos and discussing the morphologic and histologic changes and defects on mouse. The female animals were divided in three groups. The first group untreated as a control group but the second and third group received 10% and 20% solutions of ethanol respectively. Animals get use to certain level of ethanol solution and in the 10th day, the pregnancy period has been started. Then on the 19th day of gestation, the embryos were taken out from their mother's uterus and were examined for morphologic, histologic and skeletal disorders. In the first examination, the major defect was weight and length reduction in the second and third groups. These defects, were severe in the second group in compare to third group that might be related to little consumption of the ethanol solution, due to bitter taste. In conclusion the teratogenic effect of alcohol on skeleton and joint is clear.

Acta Medica Iranica 35 (3 & 4): 63-68; 1997

Key words: Fetal alcohol syndrome; embryo abnormalities; mouse

INTRODUCTION

Ethanol has been known as a teratogenic factor for many years but fetal alcohol syndrome (FAS) has not been fully understood until recently. Alcohol induced defects in embryos, such as malformation, immaturity and IUGR (Intrauterine growth retardation), CNS dysfunction, behavioral disorders and mental retardation, are of the most common reported anomalies from all over the world (1). Alcohol, cross the placenta easily and accumulate in the embryo (2).

It has been suggested that those newborns whose mother were addicted to alcohol express common deformities, especially on their faces, growth, mental retardation and heart dysfunction (3), skeleton disorders plus hormonal and protein insufficiency. All of the above mentioned disorders are classified as FAS (4).

MATERIALS AND METHODS

Chemicals

Ethanol; Formaldehyde; Hematoxilin, Eosin and Alizarin red were purchased from Merk (Germany).

Animals

Albino mice (25-30 g) were housed under standard conditions of food, water, constant temperatures ($22 \pm 1^\circ\text{C}$).

Experiments

Animals were divided into three groups, (15 in cages A, B, and C). The first group was control group. Animals in cages B and C received 10% and 20% solutions of ethanol respectively. The amount of consumed water in cages B and C was less than cage A at first 3 days due to the alcohol taste. When animals fell into alcohol flavour, equal volume of solution consumed in all cages. At the end of the 10th day, animals were coupled (2 female and 1 male) in each separated cage, for 14 hours. Then the female mice were checked for vaginal plaque, and separated in three cages A as control group B and C. The day that vaginal plaques were noticeable was called day 0. The weight of animals measured every day. The volume of consumed solution in each cages were measured and the container were refilled at the end of 24 hours. After 19 days the mice were anaesthetised to remove the embryos.

Totally, of 204 embryos with their placenta were found and under following procedure prepared for microscopic examination. After rinsing with saline solution they were placed in 10% formaldehyde solution for 48 hours. The weight of each embryo was measured separately and their external features and morphological aspects was studied. Histologic evaluation using H and E method were also carried out along with Alizarin red to study their bone structure.

RESULTS

At the end of the pregnancy 204 mice embryo were found and their macroscopic and microscopic observation lead to realize that death and atrophy of embryo in the uterus was more in group C in comparison with group B. Size measurement by collis of these animals showed significant reduction in group B in comparison with group C (Fig. 1). In embryos belong to group B, muscle-skeletal abnormalities, cyanosis, amelia, focomelia, skin shrinkage (Fig. 2 and 3), abnormal rotation of limbs (Fig. 4), abnormal phalange, microcephaly, malformation of nose and tail length (Fig. 5 and 6), subcutaneous hematome in head and thorax, reduction of crown rump length and crown hill length were observed and the difference with group C and control was significant ($P < 0.05$). Macroscopic

observation in with group C lead to realize that hydrocephaly, reduction of upper and lower limb length, hand and foot length, size and weight were exist and differences with group A and B were significant ($p < 0.05$). In histologic assessments, disorders such as cleft palate (Fig. 7 and 8), ocular anomalies, lowered nasal septum, pulmonary hypoplasia and hyperemia, Joint stiffness, polycytemia, adhesion between tarsus and tibial and fibula (Fig. 9 and 10), phalanx agenesis, skin thickness have been more in group B than group C than control group ($p < 0.05$).

DISCUSSION

Alcohol had been known as a teratogenic agent for many years. In 1736, a letter was sent to the British parliament by JIN epidemiologic center, stating that children born from alcoholic mothers, looked ill and were weak in general because of alcohol action on the developing fetuses (5). While not all cells or tissues are equally susceptible to alochol during similar embryogenic events, most researchers agree that altered cellular

function is a requisite for teratogenic manifestation (6). In addition, alcohol embryopathy represents direct and indirect teratogenic influences (7,8). The indirect effects includes, maternally mediated effects such as poor maternal nutrition, decreased placental transport of nutrients, hypoxia, and altered endocrine status. The direct effects of alcohol on cellular function are altering the chemoreceptor sensitivity, enzyme activity and membrane integrity (9,10,11). In this study, it was clearly noticed that the FAS cause a series of defects including ocular, neural, cardiac disorders and skeletal abnormalities such as joint anomaly, absence of phalanx and urogenital malformations. Previously it has been shown that alcohol cause microcephaly, dried and thick skin with growth retardation, delayed ossification in rat fetuses (12,13,14,15). Some disorders in hormonal and neural systems has also been observed which could be related to the reduction of protein synthesis and serotonin level in embryo's brain (16). Hydrocephaly and craniofacial malformations were also reported (17). Our results shows that alcohol has a teratogenic effect in pregnant mice especially on skeleton and joints of fetuses.

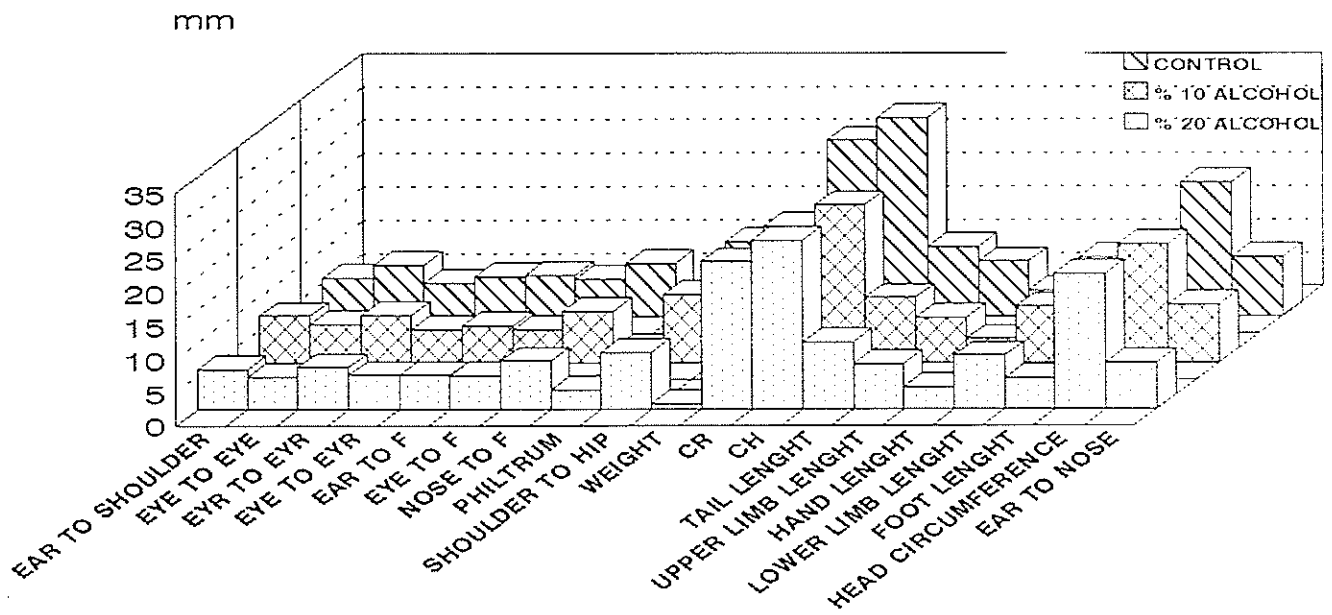


Fig. 1. Measurement of the mouse fetuses in three groups

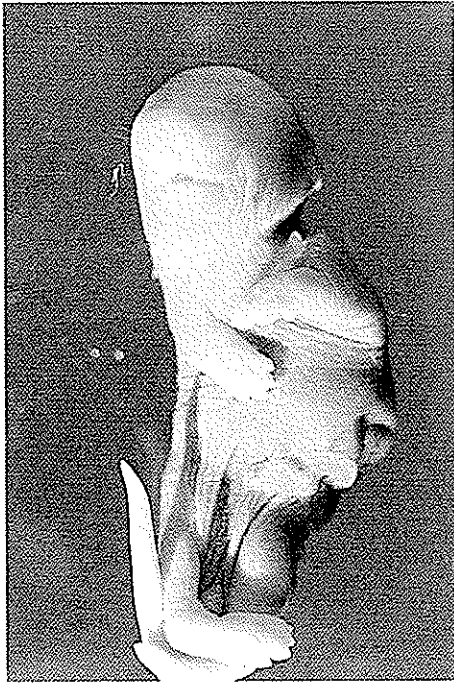


Fig. 2. Malformation in upper and lower limb. The lower limb is in adduction position and abnormal medial rotation



Fig. 3. Abnormal tail curvature and right leg shortness and adduction position of the left foot



Fig. 4. Lower limb malformation: Agenesis in right tarsal bones



Fig. 5. The 18 days mouse embryo Alisarin red staining (control group)

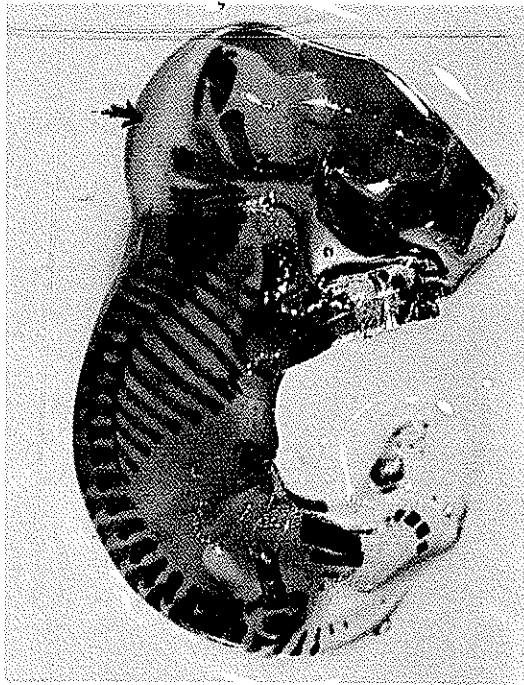


Fig. 6. Hydrocephalic embryo. Note the position of the skull bones and their articulation



Fig. 7. Frontal section through oropharynx from control group

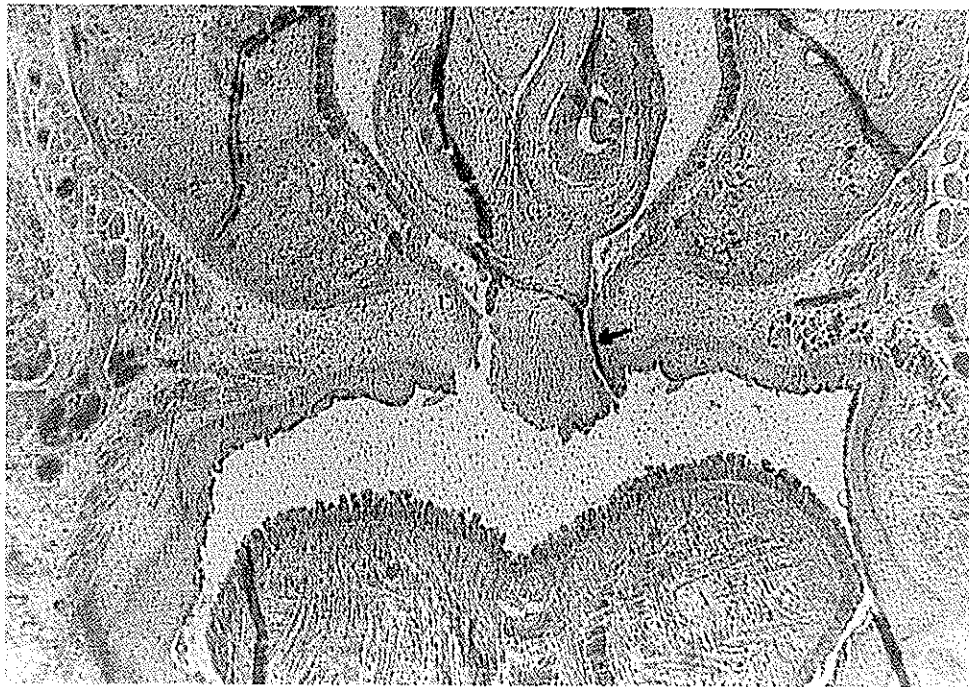


Fig. 8. Frontal section through oropharynx disunion of palatal shelves and low positioning of nasal septum



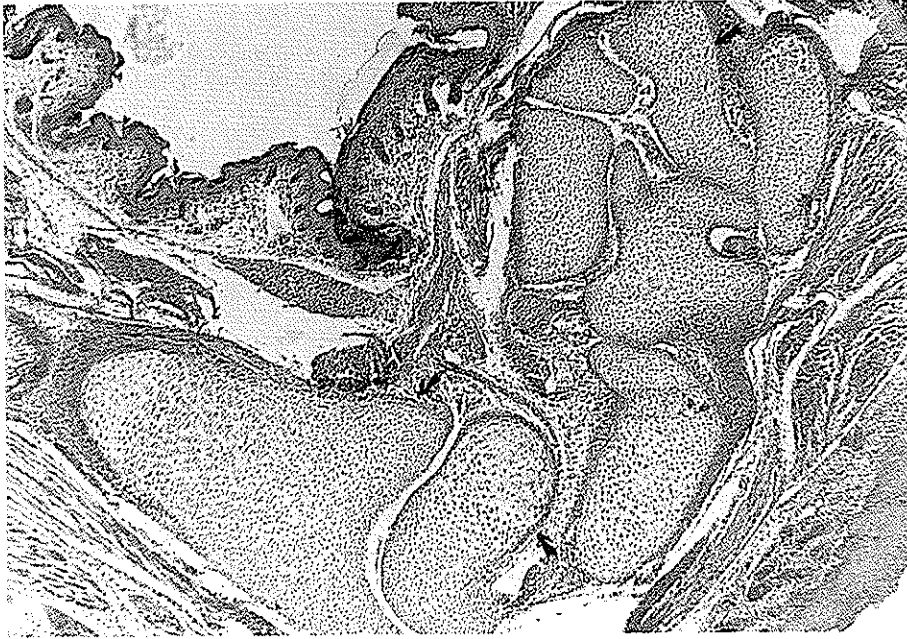
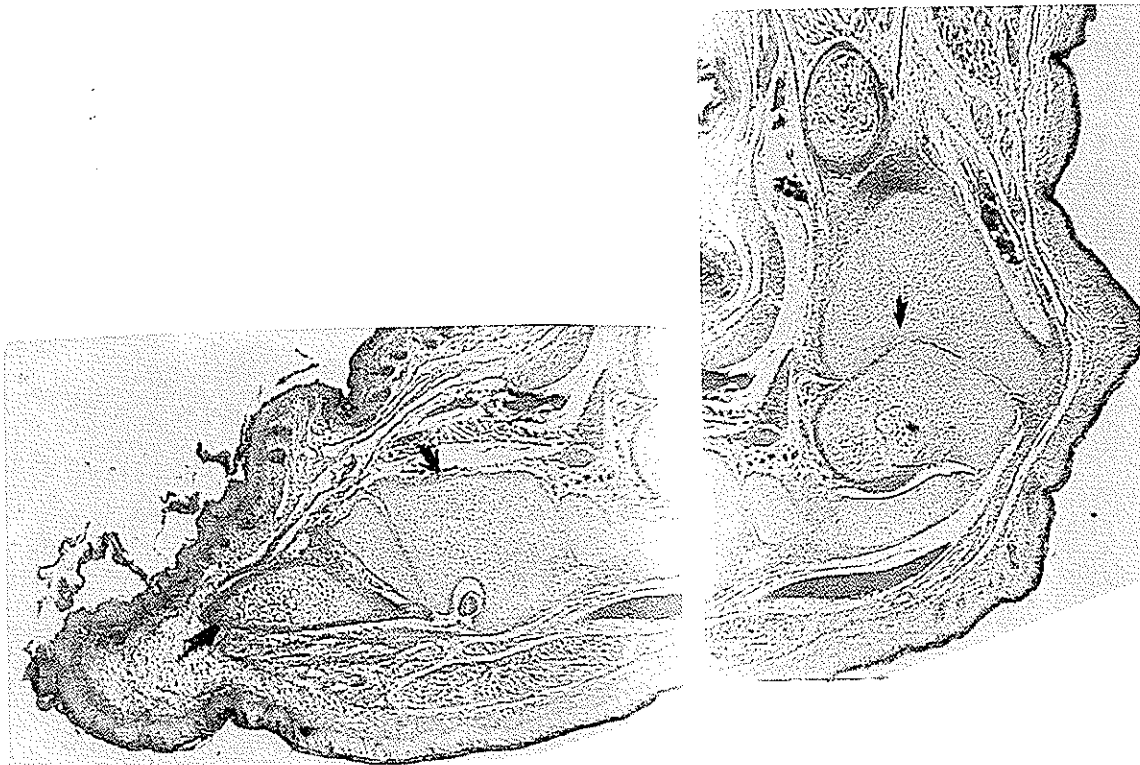


Fig. 9. Tarsal bone section of a 18 days embryo, bony adhesion and agenesis of phalanx



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Fig. 10. Saggitalis section of the leg and angle of a 18 days mouse embryo in group C (20%), shortness of foot and adhesion between tibia and talus are visible and abnormality in metatarsal bone

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