

# Teratogenic effects of Ethanol Vapour exposure on chick embryos

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## Abstract

**Objective:** To observe the effect of ethanol vapours on chick embryos regarding developmental defects and hatchability characteristics.

**Methods:** An experimental study was performed in the Department of Anatomy at the Regional Center of College of Physicians and Surgeons, Islamabad, from February, 2006 to February, 2007.

Chicken eggs after having been exposed to ethanol vapours produced in a specially designed glass chamber, were dissected on day 7, day 10 and day 22 or on hatching and compared with age-matched controls. A breathalyzer was used for monitoring level of ethanol vapours inside the incubator.

**Results:** The results show that experimental group had comparatively more cases of delayed and assisted hatchings as well as growth retardation resulting into failure of retraction of yolk sac, as compared to the controls.

**Conclusion:** Ethanol vapour exposure increases the risks of developmental defects with increasing embryonic age. Increased duration of exposure, causes delayed hatching and more assisted hatchings. Newly hatched alcohol exposed chicks showed diminished locomotor activity and poor balance.

**Keywords:** Chick embryo, Ethanol vapours, Growth retardation (JPMA 61:328; 2011).

## Introduction

Foetal alcohol syndrome (FAS) is caused by excessive maternal alcohol use during pregnancy. It is one of the major causes of preventable congenital anomalies and developmental disabilities.<sup>1</sup> It presents with growth retardation, abnormal facial features and brain dysfunction.<sup>2</sup> The full spectrum of prenatal alcohol effects is referred to as Foetal Alcohol Spectrum Disorder (FASD).<sup>3</sup>

Drinking has long been the most common type of alcohol intake for centuries. But nowadays another route is getting very popular among the western population, which is inhalation of alcohol vapours rather than drinking. For this purpose a new device, "Alcohol Without Liquid Vaporizer" has been used for inhaling vapours rather than drinking.<sup>4</sup> Companies who are manufacturing the product claim that it will allow consumers to enjoy pleasurable effects of alcohol intake without any harmful effects. But scientists are of the opinion that alcohol intake through this route will lead to direct absorption through the nose and lungs into bloodstream bypassing the stomach and liver.<sup>5</sup>

Various animal models have been used by researchers to investigate the causative factors and effects of various alcohol-related diseases. Such models have helped researchers explore the mechanisms by which both short-term and long-term drinking can interfere with normal developmental processes of the embryo. Chick embryo is a popular experimental model to study the effects of ethanol on different developmental processes in an embryo.<sup>6</sup>

Humans are also involuntarily exposed to ethanol vapours since ethanol is added to gasoline. Studies are carried out to investigate the neuromotor effects of ethanol vapour exposure on human volunteers.<sup>7</sup> This research was conducted to expose chick embryos to ethanol vapours produced in a specially designed vapour chamber. These chicks were grossly examined to see any developmental defects, along with their hatchability characteristics which included their day and mode of hatching.

## Materials and Methods

The study design of this project was experimental. Chick embryos were exposed to ethanol vapours and compared with controls. The project was carried out at Department of Anatomy, Regional Centre, College of Physician and Surgeon, Islamabad between February 2006 to February 2007.

A total of 180 'Desi' ('Desi' is a term used in the South Asian region to refer to poultry animals which are strictly fed an organic vegetarian-alone diet, without any animal or unnatural sources fed to them, thus precluding any artificial effects on the animals from artificial, blended or non-organic feeds) chicken eggs collected from Poultry Research Institute, Punjab, and Rawalpindi were divided into control group A and experimental group B of 90 eggs each. The day when eggs were placed in the incubator was taken as day 1. Each group was further subdivided into 3 subgroups based on the day of sacrifice or hatching. Subgroup 1 was sacrificed at

day 7. Subgroup 2 was sacrificed at day 10. Subgroup 3 was dissected at day 22 or on hatching whichever was earlier. Eggs which were cracked or stored in the refrigerator were excluded from the study.

Group B1 was exposed to ethanol vapours from day 1 to 6. The embryos in this group were scheduled to be sacrificed on day 7. The embryos to be sacrificed on day 10 were exposed to ethanol vapours from day 1 to 9. The chicks that were dissected either at day 22 or else on hatching, whichever was earlier, were exposed to ethanol vapours from day 1 to 9.

An incubator with capabilities of maintaining and monitoring temperature, humidity and for turning the eggs periodically was used for incubating the eggs. The temperature in the incubator was maintained at 102°F and the relative humidity was kept between 70% to 80%.<sup>8</sup>

Ethanol vapours were produced in the glass chamber

manually taken out by breaking the shells.

The day and mode of hatching of chicks was noted. Mode was either normal or assisted. The chick embryos and newly hatched chicks were examined for any gross abnormalities, abnormal gait, posture and any other abnormal behaviour as compared to the control.

Chi-square test was used for analyzing the day and mode of hatching. Percentages of newly hatched chicks with gross abnormalities in both control and alcohol exposed group were used for calculating p value by applying student t test for percentages.

## Results

1. Day of Hatching: In the present study control group A3 had significantly more hatchings on day 21 as compared to experimental group B3 which had more hatchings on day 22 (Table). In other words, experimental group B3 had more

**Table: Day and mode of hatching of control subgroup A3 and experimental subgroup B3.**

Subgroup	Day of hatching						Total
	Normal	Day 21 Assisted	Total	Normal	Day 22 Assisted	Total	
A3	23	0	23	4	3	7	30
B3	10	0	10	4	16	20	30

A3=Newly hatched control chicks.

B3=Newly hatched alcohol exposed chicks.

p-value of difference between A3 & B3 regarding the day of hatching=0.001.

p-value of difference between A3 & B3 regarding the mode of hatching=0.000.

containing ethanol into which air was bubbled with the help of an air pump.<sup>9</sup> The glass chamber was completely sealed to prevent the leakage of vapours from the chamber. The flow of air into the glass chamber was adjusted with the help of a valve which was built within the plastic tube leading from the air pump. Vapours collected in the chamber were transmitted into the incubator through a plastic pipe fitted with an adjustable clamp for controlling vapour flow. Concentration of ethanol in the incubator was maintained in the range of 0.75mg/l to 1.5mg/l. This dose was determined with the help of a preliminary project.

Ethanol vapour level in the incubator was checked and maintained by using a breathalyzer (CA 2000 of Viper technologies USA). Breathalyzer gives the blood alcohol concentration (BAC). The leaflet for the device tells that 0.01% BAC is equal to 0.05mg/l of BRAC (Breath alcohol concentration). This information was used for conversion of BAC to BRAC.

The day 7 and day 10 embryos were dissected out by cutting chorioallantoic membrane and amnion.

Some chicks hatched by day 22 while other chicks which could not hatch by themselves till day 22 were

delayed hatchings as compared to the control group A3.

2. Mode of Hatching: The mode of hatching was either normal or assisted. In normal hatching, the chick hatched by itself. In assisted hatching, the shells were manually broken and chicks were drawn out. In assisted hatchings some chick embryos were able to start the process of piping by cracking their shells but were unable to hatch out of their shells. In experimental group, assisted hatchings were more than normal



Figure-1: Newly hatched alcohol exposed chicks. The chick that was unable to stand erect and had diminished mobility is shown with an arrow.



Figure-2: An alcohol exposed chick with failure of retraction of yolk sac (YS).

hatchings as compared to control group which had less assisted hatchings (Table). The difference between the two hatchings was found to be statistically significant ( $p = 0.000$ ).

3. Gross Examination of Chicks and Embryos: The newly hatched chicks of the group B3 showed behavioral changes. A total of 33% chicks demonstrated wobbly gait, staggering, and poor balance. Whereas 56% of chicks showed diminished mobility and were unable to stand erect (Figure-1). In all 90% of newly hatched chicks of control group were active and healthy while 10% were taken out through assisted hatching and showed diminished mobility.

On gross examination of subgroup 3, it was seen that there was failure of retraction of yolk sac into the abdominal cavity in the experimental chicks (Figure-2). Yolk sac starts retracting back into the abdominal cavity at day 19 and this retraction is completed at day 20.<sup>8</sup> There was only 1 chick in subgroup A3 (out of 30) and 9 chicks in subgroup B3 (out of 30) which had failure of retraction of yolk sac. Failure of retraction of yolk sac was significantly more in experimental group B3 than that of control group A3 ( $p=0.000$ ). No gross abnormalities were seen in day 10 and day 7 embryos in both dead and living chick embryos in the experimental group.

## Discussion

1) Day and Mode of Hatching: The control group A3 had significantly more hatchings on day 21 as compared to experimental group B3 which had more hatching on day 22. In other words, experimental group B3 had more delayed hatchings as compared to the control group A3. The mode of hatching was either normal or assisted. In normal hatching, the chick hatched by itself. In assisted hatching, the chicks either failed to pip the eggshell or failed to come out after pipping the shells, again leading to a delay in hatching. In experimental group, assisted hatchings were more than normal hatchings as compared to control group which had

less assisted hatchings. The comparison between the two hatchings was found to be highly significant ( $p = 0.000$ ). The cause of delayed hatching was either dead chicks or chicks who had diminished mobility. The hypoactivity could probably have been due to the central nervous system damage after ethanol exposure for the initial 9 days of incubation. Prenatal alcohol exposure showed several structural abnormalities including reduction of brain size, prominent brain shape abnormalities with narrowing in the parietal region and reduced brain growth in portions of the frontal lobe seen through certain neuroimaging techniques. Certain areas of brain are more prone to prenatal alcohol exposure, as seen in volumetric and tissue density studies. These studies showed disproportionate reductions in the parietal lobe, cerebellar vermis and the caudate nucleus.<sup>8</sup>

Foetal alcohol syndrome is accompanied with muscle weakness, muscle wasting, and atrophy which could be another cause of hypoactivity in these chicks. David and Subramaniam<sup>9</sup> assessed the effects of prenatal alcohol exposure on the developing rat neuromuscular system by injecting pregnant Sprague-Dawley rats intraperitoneally with 1.0 ml of 20% ethyl alcohol. Unexposed rats served as controls. There was a high proportion of polyneuronally innervated endplates at the neuromuscular junction in the alcohol-exposed rats. The muscle weights, as well as the number and size of the muscle fibers, were significantly reduced in these animals showing muscle atrophy. A light-microscopic examination of the nerve sections revealed alterations in the connectivity of myelin. The finding that a higher proportion of endplates were polyneuronally innervated in the alcohol-exposed rats indicates that the maturation process of the neuromuscular system was delayed, thus confirming the deleterious effects of alcohol on growth and maturation of the nerve-muscle system leading to hypoactivity.<sup>9</sup> Myocyte atrophy and death are the main pathological findings. Pathogenic mechanisms are pleiotropic, the most relevant being disturbances in carbohydrate, protein, and energy cell turnover, signal transduction, and induction of apoptosis and gene dysregulation.<sup>10</sup>

Alcohol causes skeletal muscle atrophy and death by damaging its metabolism. Insulin plays an important regulatory role in glucose uptake and utilization in skeletal muscle. Alcohol can acutely reduce the normal metabolic responses of skeletal muscle to the action of insulin which causes acute impairment in glucose metabolism. Skeletal myopathies due to alcohol are believed to result from abnormalities in synthesis of muscle protein.<sup>11</sup>

2) Gross Examination of Chicks and Embryos: The newly hatched chicks of the experimental group showed behavioural changes. Thirty three percent chicks demonstrated wobbly gait, staggering, and poor balance.

Nancy Morris and colleagues<sup>12</sup> used a chick model in which adults chicks of 2 months age were given ethanol by diluting it in their drinking water. The birds demonstrated wobbly gait, staggering, and poor balance. The most important organ in this regard is cerebellum which is basically responsible for equilibrium and balance. Researchers have utilized quantitative structural magnetic resonance imaging (MRI) to examine the brains of living children and adults with histories of heavy prenatal alcohol exposure. These studies indicated structural abnormalities in various regions of the brain, including the cerebellum.<sup>13</sup>

In this study 56% chicks showed diminished mobility and were unable to stand erect. Another probable cause of hypoactivity could be alcohol withdrawal. Slawecki and Roth<sup>14</sup> exposed male Sprague Dawley rats to ethanol vapour for 12 or 14 days and then assessed their locomotor activity. Hypoactivity emerged rapidly in rats during ethanol withdrawal. Withdrawal induced hypoactivity was also seen in studies conducted on different species of rats after ethanol intake by other routes.<sup>15,16</sup>

In the present study experimental group B3 showed a predominant failure of retraction of yolk sac into the abdominal cavity. Normally, yolk sac starts retracting back into the abdominal cavity at 19th day and this retraction is completed at day 20.<sup>17</sup> Failure of retraction of yolk sac was significantly more in experimental group B3 than that of control group A3, which is a sign of growth retardation leading to this ventral body wall defect. In a study by Joydeep,<sup>18</sup> chick embryos were exposed to single doses of 5%, 10% and 15% ethanol, and the effects on general growth and development of these chicks were studied. There was significant growth retardation found in these chicks which is in accordance with this study. In another study chick embryos were explanted in shellless cultures and single dose of 50% ethanol was applied. Ethanol significantly increased the mortality rate and induced growth retardation.<sup>19</sup> Experimental studies on other species show that apoptosis, oxidative stress, altered cell cycle, suppressed DNA and protein synthesis are some of important causes of ethanol induced deformities.<sup>20,21</sup> No gross abnormalities were seen in day 10 and day 7 embryos in both dead and living chick embryos in the experimental group which shows that both increase duration of exposure and increasing embryonic age enhances the risk of growth retardation and developmental defects in developing chick embryos.

### Conclusion

Ethanol vapour exposure increases the risks of

developmental defects with increasing embryonic age and increased duration of exposure. Hatching is delayed and there are more assisted hatchings. Newly hatched alcohol exposed chicks showed diminished locomotor activity and poor balance which may be attributed to damage to central nervous system or skeletal muscle.

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