

# Effect of Ethanol Vapour Exposure on Survival of Chick Embryos

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## ABSTRACT

**Objective:** To determine the survival of chick embryos after ethanol vapour exposure by noting the number of dead and alive embryos and comparing with age-matched controls.

**Study Design:** Experimental study.

**Place and Duration of Study:** Department of Anatomy at the regional centre of College of Physicians and Surgeons, Islamabad, from February 2006 to February 2007.

**Methodology:** Chicken eggs, placed in an incubator, were exposed to ethanol vapours produced through a specially designed vapour chamber. The experimental group was dissected on day 7, day 10 and day 22 or hatching whichever was earlier and compared with age-matched controls. The proportion of ethanol vapours in the incubator was monitored with a breathalyzer.

**Results:** No statistical difference was seen in the survival of day 7 alcohol-exposed embryos and their age-matched controls. The survival of day 10-control embryos was significantly higher than alcohol exposed group of same age. The embryos exposed to ethanol vapours from day 1 to day 10 and then followed till hatching or day 22, whichever was earlier, had significantly lower survival than age-matched controls.

**Conclusion:** In this study, ethanol vapour exposure decreased embryo survival with increasing embryonic age and increased duration of exposure.

**Key words:** Chick embryo. Ethanol vapours. Survival.

## INTRODUCTION

Alcohol, despite being toxic to humans, is used in industry and home for the manufacture of compounds like gasoline additives, paints and perfumes.<sup>1</sup> In general usage, alcohol refers almost always to ethanol, also known as grain alcohol. Ethanol is a colourless, inflammable compound, which is the component of alcohol containing beverages like wine, spirits and champagne. Ethanol is also used as a solvent in medicines, perfumes, and vegetable essences, such as vanilla. It is also used as a topical antiseptic.

Alcohol intake during pregnancy can lead to fetal abnormalities.<sup>2</sup> Prenatal alcohol exposure exerts teratogenic effects on the developing fetus, mainly manifest by growth retardation, abnormal facial features and Central Nervous System (CNS) damage, collectively known as the Fetal Alcohol Syndrome (FAS). Alcohol also causes cardiovascular anomalies.<sup>3</sup>

Though, the major route of ethanol intake is by drinking, another route now being used in the western world is

inhalation. Ethanol can be snorted into the nose or inhaled into the mouth through a tube using a new device known as an Alcohol Without Liquid Vaporizer (AWOL), which allows users to feel the effects of alcohol by inhaling vapours rather than drinking. Studies are also being done on humans to see the neuromotor effects of ethanol vapour inhalation as ethanol is also added to gasoline, increasing the involuntary human exposure to this chemical.<sup>4</sup>

Chick embryo is a popular experimental model for developmental studies. It has certain practical advantages like easy experimental manipulation, rich history in developmental biology and the short incubation time, which emphasize the importance of the chick embryo, as a model organism for developmental studies.<sup>5</sup>

Although the harmful effects of ethanol ingestion have already been established, the effects after ethanol inhalation are still being worked up.<sup>6</sup> Keeping all this in mind, this research was conducted to expose chick embryos to ethanol vapours, produced in a specially designed vapour chamber, to determine the number of dead and alive embryos and compare it with age-matched controls.

## METHODOLOGY

It was an experimental study. The project was carried out at the Department of Anatomy, Regional Centre, College of Physicians and Surgeons, Islamabad between February 2006 and February 2007.

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A total of 180 local chicken eggs taken from Poultry Research Institute, Punjab, Rawalpindi, were divided into control group (A) and experimental group (B) of 90 eggs each. Each group was further subdivided into three subgroups (1, 2, and 3), based on the day of sacrifice, containing 30 eggs each. The day when eggs were placed in the incubator was taken as day 1. Subgroup 1 was sacrificed at day 7, subgroup 2 was sacrificed at day 10 and subgroup 3 was dissected at day 22 or on hatching, whichever was earlier. The eggs which were cracked or stored in the refrigerator were excluded from the study.

Group B 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 only from day 1 to day 9.

A purpose-built incubator, with capabilities for maintaining and monitoring temperature and humidity and turning the eggs periodically, was used for incubating the eggs (Figure 1). The temperature in the incubator was maintained at 102°F and the relative humidity was kept between 70-80%.<sup>7</sup> The level of ethanol vapours was brought to the required range inside the incubator before the placement of experimental eggs.

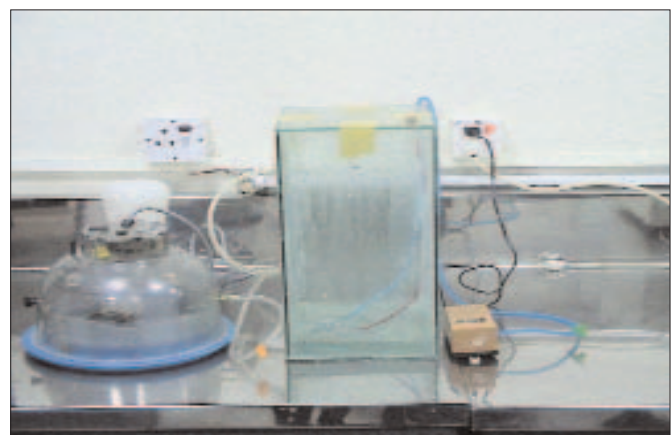
For the production of ethanol vapour, a special apparatus was designed, which consisted of a glass chamber containing ethanol into which air was bubbled with the help of an air pump (Figure 1). The glass chamber was completely sealed to prevent the leakage of vapours from the chamber. Vapours collected in the chamber were transmitted into the incubator. 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. Vapour transmission into the incubator was controlled with the help of an adjustable clamp. Initially, 20% ethanol was poured in the glass chamber. Later, the glass chamber was re-filled on regular basis with higher grades of ethanol, even absolute ethanol so as to maintain the level of ethanol vapours in the egg incubator within the given range. The reason for using higher grades of ethanol was that over a period of time, ethanol in the chamber evaporates earlier than water as it is more volatile. Concentration of ethanol in the incubator was maintained in the range of 0.75-1.5 mg/l determined by a preliminary project.

The level of ethanol vapour was checked by taking regular measurements of alcohol content using a breathalyzer (Figure 2). Breathalyzer is a digital alcohol detector, which is used by traffic police to detect Blood Alcohol level/concentration (BAC) indirectly by measuring breath alcohol in suspected drunken drivers.

As per manual, 0.01% BAC was equal to 0.05 mg/l of Breath Alcohol Concentration (BrAC). This information was used for conversion of meter reading to actual concentration of alcohol in the air samples obtained from the incubator. For the purpose of measuring the ethanol concentration levels in the air of the incubator using this breathalyzer, air was sucked with the help of a syringe from the incubator and it was pushed into the inlet pipe of the breathalyzer. After 5 seconds, breathalyzer gave the “blood alcohol concentration” reading which was converted to breath alcohol concentration with the help of the conversion equation already given.

The day 7 and day 10 embryos were dissected out from the eggs on their respective days (Figure 3). Chorion-allantoic membrane and amnion were cut and the embryo was taken out by removing albumen and yolk. Some chicks hatched by day 22. Other chicks which could not hatch till day 22 were manually taken out by breaking the shells.

Data collected was fed in the computer and SPSS version 10 was applied. Chi-square test was used for analyzing the data. The variable fed into the data analysis software was termed as ‘survivability’ where a value of ‘1’ denoted alive embryo and a value of ‘2’ denoted dead embryo.



**Figure 1:** Experimental apparatus showing an incubator for placing eggs (1), glass chamber for ethanol vapour production (2), an air pump for bubbling air into the glass chamber (3), clamp for controlling vapour transmission into the incubator (4), a valve for controlling airflow from the air pump to the glass chamber (5).



**Figure 2:** Breathalyzer.



**Figure 3:** Day 7 and day 10 control chick embryos.

## RESULTS

Group B3 was exposed to ethanol vapours from day 1 to day 9 and those embryos were allowed to develop till hatching or day 22, whichever was earlier. The survival of the control group A3 was significantly higher than that of the experimental group B3 ( $p = 0.001$ , Table I). Out of 30 chicks in group B3, total dead chicks were 9, out of which 6 chicks were well-formed and 3 embryos were macerated. There was no dead chick in control group A3.

Group B2 was exposed to ethanol vapours from day 1 to day 9. The survival of group A2 was significantly higher than that of experimental group B2 ( $p = 0.004$ , Table I). The total dead embryos in group B2 were 8, out of which 1 embryo was well-formed, 5 embryos were macerated and 3 were only macerated blastoderms.

Group B1 was exposed to ethanol vapours from day 1 to day 6. There was no statistically significant difference in the survival of experimental group A1 and control group B1 ( $p = 0.313$ , Table I). There were no dead embryos in control (A1) group. There was only 1 dead embryo (out of 30 embryos) in the experimental group B1, which was a macerated blastoderm.

Overall, the survival of control group A (98.8%) was significantly higher ( $p < 0.001$ ) than experimental group B (80%).

**Table I:** Percentage of dead and alive embryos in alcohol exposed and control subgroups.

Subgroup	n	Percentage of alive embryos	Percentage of dead embryos	p-value
A3	30	100%	0%	0.001
B3	30	70%	30%	
A2	30	96.6%	3.33%	0.004
B2	30	73.3%	26.6%	
A1	30	100%	0%	0.313
B1	30	96.6%	3.33%	

## DISCUSSION

Alcohol is known to exert significant adverse effects on liver, brain, heart, skeletal muscle, pancreas, hematological system, immune system, gastrointestinal apparatus and endocrine system. Chronic alcohol abuse induces multiorgan deterioration with an increase in mortality.<sup>8</sup>

In the present study, day 7 experimental group B1 was exposed to ethanol vapours from day 1 to day 6. There was no significant statistical difference in the survival of experimental group B1 and control group A1. Day 10 experimental group B2 was exposed to ethanol vapours from day 1 to day 9. The survival of control group A2 was significantly higher than that of experimental group B2. This is in accordance with one of the previous studies in which mice were subjected to alcohol intoxication by adding alcohol to drinking water. Survival

was significantly lower in the alcohol-exposed mice with increased duration of intoxication.<sup>8</sup> In another study, Joydeep injected ethanol in the air-sac of white leghorn chick eggs and found marked prenatal mortality.<sup>9,10</sup> This was in accordance with the previous studies done on chick embryos, which showed decrease in survival after ethanol exposure with increasing embryonic age. Ruckman and colleagues injected alcohol in chick embryos at different stages and observed decreased survival with increasing embryonic age.<sup>11</sup> Randall and Anton found an increase in both prenatal mortality as well as birth defects after oral alcohol administration of alcohol on gestation day 10 in C57BL/6J mice.<sup>12</sup> Insignificant statistical difference in survival of alcoholic and control chick embryos at day 7, in the present study, could be attributed to less embryonic age and less duration of exposure. However, day 10 alcoholic chick embryos had less number of survivors, which depicted increased mortality vis-à-vis embryonic age as well as more duration of exposure.

Experimental group B3 was exposed to ethanol vapours from day 1 to day 9 and then these embryos were allowed to develop till hatching or day 22, whichever was earlier. The survival of control group A3 was significantly higher than that of experimental group B3. One of the reasons of increased mortality at hatching could be due to the fact that ethanol causes decreased survival with increasing embryonic age.<sup>11</sup> Increased mortality at hatching could also be due to the damage already done by alcohol exposure during initial 9 days of incubation. It was previously seen that even after alcohol withdrawal, patient had poor prognosis because of the already established pathologies due to alcohol exposure. Digennaro and colleagues found that previous heavy alcoholism, in spite of long-term withdrawal, was associated with endothelial dysfunction and a wide cluster of haemodynamic, vascular and metabolic abnormalities that indicate an unfavourable cardio-vascular and metabolic risk profile even in apparently disease-free former alcoholics.<sup>13</sup>

Secondly, ethanol was withdrawn at day 10 and it was seen in previous studies that alcohol withdrawal increased morbidity and mortality as withdrawal patients develop neuropsychiatric problems like delirium tremens. Delirium tremens is recognized as a potentially fatal and debilitating complication of ethanol withdrawal.<sup>14</sup> Cuculi and colleagues conducted a study on the data of previously hospitalized patients in which it was observed that patients with alcohol withdrawal develop cardiovascular problems including arrhythmias, ventricular tachycardia and atrial fibrillation.<sup>15</sup>

## CONCLUSION

Ethanol vapour exposure decreased chick embryo survival with increasing embryonic age and increased duration of exposure.

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