Continued Exposure to Alcohol and its Effect on the Development of Zebrafish Embryos

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Abstract

This experiment was conducted in order to verify past research on Fetal Alcohol Syndrome (FAS) and the developmental issues involved with it. To test the effects of alcohol on developing babies, zebrafish embryos were placed in varying concentrations of alcohol between fertilization and four days post fertilization. The embryos were examined every twenty four hours. Notable results included the physical deformation, general inactivity, and the rapid development of the alcohol-affected embryos. All of these are similar to defects found in alcohol-affected human embryos. These results provide more evidence to support the arguments of past researchers in the area of FAS.

Introduction

Ethanol is the primary type of alcohol found in alcoholic drinks. It is a neurotoxin and a psychoactive drug. At BAC (blood alcohol concentration) above 1 g/L, alcoholic beverages can cause slowed cognition, poor motor function, unconsciousness, and death (O’Neil, 2012). Zebrafish are suitable models for running tests on development because they are fairly simple organisms, they develop within days, they are similar to humans (in that they have a backbone, a nervous system and a circulatory system), and they develop outside the mother as a transparent embryo. Being exposed to alcohol during development causes Fetal Alcohol Syndrome (FAS). Embryos heavily exposed (heavy exposure is described as greater than or equal to one drink per day of pregnancy) to alcohol during development have been known to have birth defects including stunted growth, premature birth, deficiencies in the central nervous system and ocular abnormalities. Some deficiencies in the central nervous system include reduced size of the frontal lobe and cerebellum, lack of white matter which connects the two hemispheres of the brain, and displacement of the vermis, a part of the cerebellum. Most FAS side effects involve cell death, which leads to localized inflammation (Goodlet, 2001).

This experiment is not meant to find new information on the subject, but to verify the results of other research into fetal alcohol abuse. It was hypothesized that the zebrafish embryos affected by alcohol would have developmental deformations, have trouble with movement, and would not live as long into development as the control. The experiment found that the alcohol-affected embryos developed more quickly than the control, but with abnormalities in the backbone, heartbeat and coloration.

Materials and Methods

Materials

- One bottle of Instant Ocean/ Embryo media
- One bottle of 30 mM ethanol solution
- One bottle of 100 mM ethanol solution
- One bottle of 300 mM ethanol solution
- One beaker for dead embryos and waste solution
- One permanent marker
- One minimum bore 1.5mm pipette for embryo transferring to and from container
- One 1 mL pipette
- One plate with wells
- One incubator set at 28.5 degrees Celsius
- Depression slides with cover slips
- One dissecting microscope
- One compound microscope
- Zebrafish embryos
Methods

The first well in the plate was filled with one mL of embryo media using a wide bore pipette, the second well with one mL of the 30mM ethanol solution, the third well with one mL of the 100mM ethanol solution, and the fourth well filled with one mL of the 300mM ethanol solution. Ten embryos were placed in each well using the minimum bore pipette. The exact number of embryos in each well was recorded using the dissection microscope. Dead embryos were discarded into the waste beaker. Embryos were observed in the plate using the dissection microscope and individually on depression slides under the compound microscope. Observations were recorded on the students’ data sheets. The embryos were returned to their respective wells in the plate and the plate was placed in the incubator at 28.5 degrees Celsius. On days two and three the plates were removed from the incubator for observation. The dead embryos were placed in the waste beaker. The solutions were drained and replaced with new solutions of the appropriate concentrations of ethanol. Old solution was discarded into the waste beaker. Remaining embryos were counted as well as hatched fish. The embryos in each well were observed under the dissection microscope, with all observations being recorded on the students’ data sheets. Individual embryos were placed on depression slides and observed under the compound microscope. All embryos were returned to their wells in the plate and the plate was returned to the incubator at 28.5 degrees Celsius. On day four the plate was removed from the incubator and the solutions were drained. All solutions were replaced with 1 mL of the appropriate ethanol concentration. Dead embryos were removed from each well and put into the waste beaker. The embryos and fish were counted. Any observations were made and recorded on the data sheets. The embryos and fish along with their solutions were removed from the plate and put in the waste beaker. The embryos were euthanized by freezing at the end of day four.

Results

The experiment was conducted in order to verify previous research and experimentation involving FAS, alcohol abuse, and parental alcoholism impacts on a child’s development. It was hypothesised that the fish affected by alcohol would have trouble moving, developmental deformities, and would not live as long as the control fish. The embryos were separated into wells on a plate, with the control in embryo media, and others in successively denser concentrations of ethanol, the independent variable being the concentration of ethanol, and the dependent the number and developmental condition of the live fish.

The alcohol-affected embryos generally hatched earlier than the control, and earlier than expected for zebrafish. (Alcohol-affected embryos hatched starting at 48 hours post fertilization). The alcohol-affected fish did develop more quickly, growing patches of color and eye spots sooner, but had visible deformations in the backbone, eyes and heartbeat. One clearly visible deformation is the thin backbone and tail of the embryos in the 300Mm alcohol compared to that of the control on day two. Another difference between the control and embryos in 300Mm alcohol on day two is that the yolk sack is visibly larger on the 300Mm alcohol-affected embryos. Most development in the alcohol-affected fish slowed to nearly a stop by 72 hours post fertilization, and the alcohol-affected fish stopped gaining color after the initial presence of color at 24 hours post fertilization. The heartbeat of the alcohol-affected fish was slower than the controls’, and was not at regular intervals. The alcohol-affected fish were also inactive and rarely moved even after hatching.

The results supported the initial hypothesis, as well as providing further detail about alcohol’s impacts on development. Most of the desired information was found in the results, although zebrafish cannot provide information on cognition, mental disability, and societal issues which FAS and alcoholism can be a cause of. One problem encountered during the experiment was the death of all embryos in the first run of the experiment. The solutions in the wells were not changed well enough and protists grew in the solutions,
eating all the embryos by day three. The experiment was conducted again with better precautions taken to protect the fish from their external environment. Specifically, a greater amount of methyl blue was added to the solution. No protists appeared during the second experiment.
Discussion

Some notable results include the premature development, general inactivity, physical deformations, and lack of color in the alcohol-affected fish. The deformations could be related to the rapid development of the alcohol-affected fish. The rapid development and premature hatching of the alcohol-exposed zebrafish embryos could be related to premature birth in human children, which is a consequence of FAS. The fact that more embryos hatched prematurely in higher concentrations provides more solid evidence that alcohol is the cause of the premature hatching.

It is possible that the alcohol-exposed embryos appeared to develop more deformities because of handling and moving by the experimenters. This is unlikely though, because the deformities noted in the alcohol-affected fish were more severe and complex than one would expect from constant handling and moving and because the control embryos were handled daily as well as the alcohol-affected embryos. The embryos being handled by the pipettes and put on slides would likely not cause irregular heartbeat, lack of color or inactivity.

The results support previous research conducted on FAS and alcohol. The experiment showed that alcohol leads to physical deformities, movement issues, premature birth and heart problems, all of which have been linked to alcohol in previous research. The results also provide further evidence that the concentration of alcohol may affect the outcomes of FAS, as problems seen became more severe in higher concentrations of alcohol.

This experiment was not entirely conclusive on the issue of FAS, and further research should be done on the societal and mental impacts of alcohol on children and adults. As embryos, zebrafish are not suitable models to test mental or societal issues, but other subjects, such as feeder mice, are better for testing these. Further research should also be done on the lack of pigmentation of the alcohol-affected fish, as further research could explain these results more accurately than this experiment.

References
