The Effect of Ethanol on Zebrafish Development

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Abstract

This experiment was designed to investigate the effects of ethanol on zebrafish embryos, in order to predict the effect of ethanol on human embryos. The zebrafish embryos were exposed to ethanol at concentrations of 0.03%, 0.1%, and 0.03%, as well as a control treatment as a means of comparison. The embryos were observed for 120 hours post fertilization. The findings showed a 70% (+/- 15.3%) difference in percent of zebrafish surviving after 24 hours between the control treatment and the 0.3% ethanol solution. Statistically significant evidence was also found to support that zebrafish exposed to ethanol were less likely to hatch. Those embryos that did survive and hatch appeared more likely to have spinal deformities. Due to the similarities between zebrafish and human embryonic development, these findings could support the theory that alcohol consumption by pregnant women could have negative effects on the fetus. More studies would need to be done, with more trials and different concentrations, to specify the exact effects as well as specifying which concentrations would cause harm.

Introduction

Ethanol & FASDs

Ethanol use during pregnancy significantly increases the probability of miscarriage or birth defects, yet 7.6% of pregnant women in the US report drinking alcohol throughout their entire pregnancy (Bernstein & Vorgias, 2019). Ethanol is more dangerous to fetuses than adults because, when introduced during fetal development, it injures organ systems as they are still growing (Brien & Smith, 1991). The effect of ethanol on surviving fetuses is known as a range of disorders called Fetal Alcohol Spectrum Disorders, or FASDs (Bernstein & Vorgias, 2019). These disorders affect about 2.4-4.8% of the population of the US (Bernstein & Vorgias, 2019). The most severe FASD is known as Fetal Alcohol Syndrome, or FAS (Bernstein & Vorgias, 2019). FASDs include three categories of symptoms, including decreased size, abnormal facial features, and disorders of the central nervous system (Bernstein & Vorgias, 2019).

Why Zebrafish?

Zebrafish are easier to use than many other organisms that are also similar to humans. (Burke, 2016). They are small, easy to care for, and develop over a relatively short period of time (Burke, 2016). Furthermore, their eggs develop outside of the mother's body and the eggs are translucent, so different developmental checkpoints can be observed easily (Burke, 2016). Finally, their genome is relatively similar to humans' so findings are often applicable to humans (Burke, 2016). Due to all of these reasons, many researchers use zebrafish in preliminary studies (Burke, 2016), which helped this experiment use previous research as a baseline for setup and comparison.

Hypothesis:

As the amount of ethanol increases, the percentages of surviving and hatching zebrafish will decrease.

Materials & Methods

Materials

- 91 zebrafish embryos
- 1 3x4 well plate with lid
- Fine tipped pipettes
- Wide tipped pipettes
- 3 glass bottles
- 100 mL graduated cylinder
- 10 mL graduated cylinder
- Dissection microscope
- 500 mL waste beaker
- 350 mL instant ocean solution
- 43 mL ethanol
- Incubator
- White paper for photo background
- Camera
- Paper and pen for data table

Procedure Summary

1. About 10 zebrafish embryos are placed into each well of a 3x4 well plate
2. The three wells in column A are filled with 0.3% ethanol solution
3. The three wells in column B are filled with 0.1% ethanol solution
4. The three wells in column C are filled with 0.01% ethanol solution
5. The three wells in column D are filled with Instant Ocean solution
6. Zebrafish are observed and counted every 24 hours for a 120 hour period, and wells are cleaned out daily and replaced with fresh solution

Results

Survival Rate: Ethanol at a concentration of 0.3% from 24 hpf to 120 hpf does decrease likelihood of survival for zebrafish embryos.

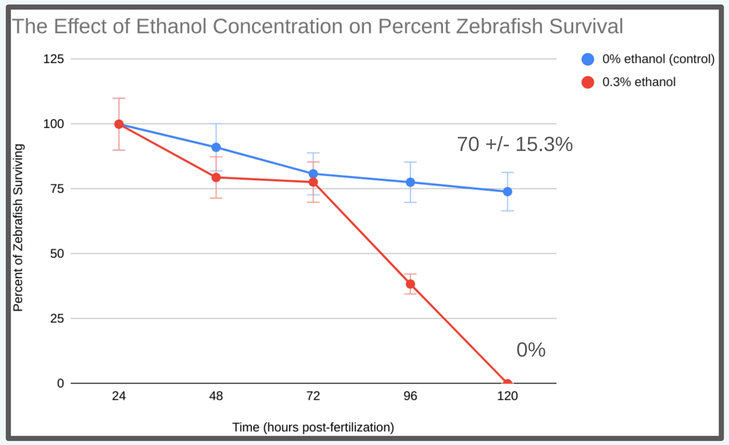

Figure 1: n = 36-60 embryos per treatment (Experiment was repeated, as results were shared with Julien & Trinker using the same procedure). Embryos "survived" if they did not appear fully opaque under the dissection microscope. Data at 120 hpf is statistically significant (p = 0.0102) (GraphPad, 2019).

Percentage Hatched: Ethanol at a concentration of 0.3% does decrease percentage of zebrafish able to hatch.

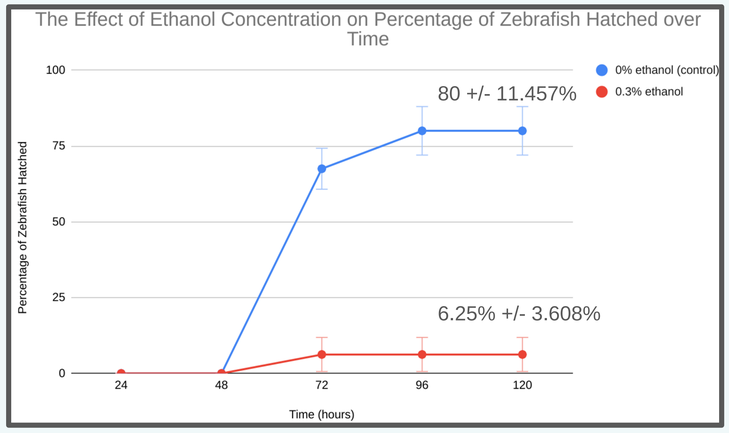

Figure 2: n = 36-60 embryos per treatment (Experiment was repeated, as results were shared with Julien & Trinker using the same procedure). Embryos "hatched" after they fully exited the outer shell. Data at 120 hpf is statistically significant (p = 0.0009) (GraphPad, 2019).

Birth Defects: Zebrafish with spinal bends, also known as scoliosis, were only found in the 0.03% and 0.1% ethanol wells. No abnormalities were found in hatched zebrafish in the control wells.

Figure 3:
Hatched zebrafish (96 hpf) in 0% ethanol solution with a straight (wild type) spine.

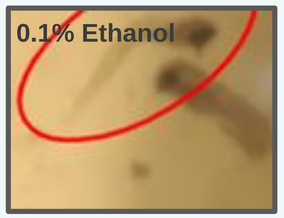

Figure 4:
Hatched zebrafish (96 hpf in 0.1% ethanol solution with a 90 degree curve in the spine

Discussion

Importance of the Findings

This data, which was statistically significant (Graph Pad, 2019), supported the hypothesis, indicating that survival and hatching of zebrafish after 96 hpf are lower when exposed to ethanol. Furthermore, birth defects were observed in the fish in lower concentrations, suggesting that ethanol also causes scoliosis in zebrafish. More specific data on percent of zebrafish with scoliosis, as well as degrees of spinal bend, would need to be recorded in order to test statistical significance. These results mirror the effects seen on humans exposed to ethanol while developing. These results reflect the 2011 experiment conducted by Ali et al., which showed that when exposed to ethanol at the earlier stages of development, the mortality rate of zebrafish increased to 88% and they also reflected similar symptoms as FAS in humans. This zebrafish model can be used to reflect human survival, providing more insight into the higher infant mortality rates and higher rates of FAS in communities with a higher prevalence of alcohol (GBD, 2018).

Relation to Humans

Although decreased concentrations of ethanol resulted in decreased negative effects, as little as 1 drink per day causes a significant increase in likelihood of FAS (Committee, 2000). Fetuses exposed to lower concentrations of alcohol are also more likely to develop FASDs than FAS, which still harm social behavior and physical health (Bernstein & Vorgias, 2019). There is no level of alcohol consumption in pregnant women that is confirmed to be safe for the fetus, so the American Academy of Pediatrics recommends abstaining from all alcohol consumption during pregnancy (Committee, 2000).

The results of exposing zebrafish embryos to ethanol showed that ethanol is deleterious to fetal development, causing decreased survival. Those that survive will have decreased quality of life, and the effect of ethanol on zebrafish mirrors FAS, including decreased development of the central nervous system, abnormal structural growth, and decreased growth rate (Ali et al., 2011). More research is needed to confirm how ethanol affects zebrafish and to connect that to its effects on humans, but it is clear from the results of this experiment that ethanol harms embryonic development in zebrafish, and exposure should be avoided during human fetal growth. This research can help to spread awareness of FAS as well as inform scientists about effects and mechanisms of FAS, which could potentially lead to better prevention and treatment for infants exposed to ethanol in the womb.

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