The Impact of Ethanol on Developing Zebrafish

By Elise Allen

SEPA SCIENCE EDUCATION PARTNERSHIP AWARD
SUPPORTED BY THE NATIONAL INSTITUTES OF HEALTH

Abstract

Each year, hundreds of gallons of alcohol are consumed by Americans, including pregnant women. This study aims to discover the effects of exposure to ethanol on development, using Zebrafish as a human stand-in. The study hypothesized that exposure to ethanol would lead to a higher mortality rate and visible deformations. One group of Zebrafish were exposed to 0.03% ethanol, and their appearance and behavior was compared to a group of Zebrafish in regular embryonic solution. This study found a correlation between exposure to ethanol and deformations, but found no trend between ethanol and a higher mortality rate. The findings may be useful in discovering the exact relationship between ethanol and development and finding better treatments for those with FASDs.

Introduction

Zebrafish (*Danio rerio)* are an ideal test subject due to the fact that they share 70% of our genes. In addition, zebrafish share many organs with humans, including the brain, pancreas, heart, intestine, eyes, bone, liver, kidney etc. (Burke, 2016). The clear egg shells also allow for better viewing of the embryo (Burke, 2016). This study chose to observe the effect of ethanol, due the prevalence of drinking in the United States. Drinking alcohol during pregnancy can result in fetal alcohol spectrum disorders, or FASDs. FASDs can result in problems with vision, hearing, heart, kidney, bones and other issues (CDC, 2018). This study hopes to better understand the effects of ethanol on prenatal development, and perhaps lead to the development of medication to specifically treat FASDs. For this study, zebrafish embryos were placed in 0.03% ethanol and their development, survival and hatch rate were compared to zebrafish embryos placed in pure Instant Ocean solution. The lab hypothesis predicted the zebrafish exposed to the ethanol solution would have a lower survival rate and visible deformations.

Materials

- 12 welled falcon plate
- 3 beakers
- Instant ocean solution
- 0.03% ethanol solution
- Incubator
- Compound microscope
- Transfer pipettes (Petering, Berg, Tomasiewicz, et. al, 2018).

Methods

The independent variable of this lab was the presence of 0.3% ethanol in the Instant Ocean solution, the dependant variable was the number of Zebrafish that survived and hatched in each set of wells, and the control group were the fish placed in pure Instant Ocean solution. The experimental group were the fish placed in 0.03% ethanol, and the sample size was approximately 120 Zebrafish. The controls included the use of Instant Ocean solution, incubation at 28.5℃ and the removal of dead embryos and waste product every 24 hours for the 96 hours post fertilization. In addition, the embryos were placed in the wells at 24 hours post fertilization.

Procedure:

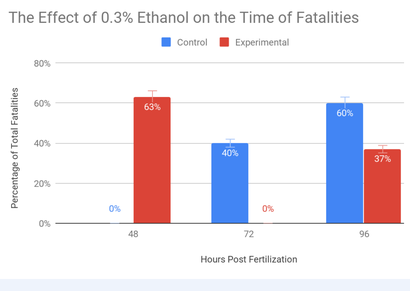
1. Fill the control and experimental wells with their proper solution
2. Place approximately 10 zebrafish in each well
3. Record number of live and hatched zebrafish
4. Remove dead fish and waste product
5. Return falcon plate to incubator
6. For the next 3 days replace solution in each well and repeat steps 3-5

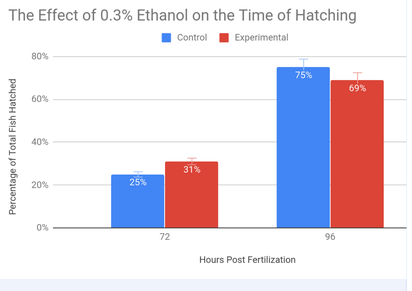
Results, Graphs and Photos

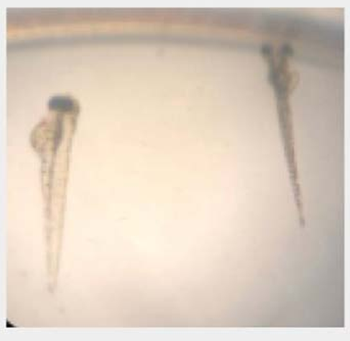
The results showed a trend between the hatching/survival rate and the presence of ethanol. The experimental group which was exposed to 0.3% ethanol, had a greater survival and hatch rate, than that of the control group, which was kept solely in Instant Ocean solution. In addition, this study found a correlation between exposure to ethanol and deformations, as seen below. The two-tailed p-value for the survival rate was found to be 0.0183.

The Effect of Ethanol on the Survival and Hatch Rate of Zebrafish

	Control	Experimental
Survival rate	52%	75%
Percent hatched from total eggs (PHE)	39%	55%
Percent hatched from alive (PHA)	75%	74%







Control group Zebrafish - 96 hpf

Experimental group Zebrafish - 96 hpf
Premature hatching

Experimental group Zebrafish - 96 hpf
Enlarged yolk sac and heart

Experimental group Zebrafish - 96 hpf
Curved spine

Conclusion

Importance of the Topic

The hypothesis at the beginning of this experiment was that exposure to ethanol would lead to a decreased survival rate and visible deformations in the zebrafish. However, the results of this study were mixed. This study found a correlation between exposure to ethanol and a greater survival rate, as seen in **fig. 6**, which does not support the hypothesis. One possible explanation for this is ethanol's bacteria killing properties. In the control wells, there appeared to be bacterial growth, which may have contributed to some control fish mortalities. As seen in **fig. 8**, the majority of the fatalities in the experimental wells occurred in the first 24 hours of being exposed to ethanol. It is possible that the experimental fish that survived the first crucial 24 hours in the ethanol solution had a better chance of survival due to ethanol's ability to kill off bacteria. However, as seen in **fig. 1, 3-5**, there was a trend between exposure to ethanol and deformations, which supports the hypothesis. The experimental fish suffered from enlarged yolk sacks, curved spines and enlarged hearts. Many of the experimental fish struggled to swim and twitched uncontrollably. In addition, there appeared to be a correlation between exposure to ethanol and premature hatching, as several of the newly hatched experimental fish were not as developed as their control counterparts. All of these issues would drastically decrease the chances of long term survival. This leads this study to infer that exposure to ethanol leads to impaired and/or mutated development and a decreased chance of survival.

Importance of the Findings

As seen in the Background Section, the results of exposure to ethanol during pregnancy can be disastrous. With a terrifyingly high percentage of pregnant women drinking alcohol, it is imperative that society becomes more aware of ethanol's impact and how to treat it. Understanding ethanol's impact can bring awareness, and hopefully decrease the number of pregnant women consuming alcohol. In addition, as seen in **fig. 1, 3-5**, there is a correlation between ethanol exposure and birth defects. Overall, further study of the correlation between ethanol exposure and deformities is important to better understand FASDs and to hopefully find better treatments.

Sources of Error:

As with any study, there were possible sources of error, such as bacterial growth in the wells. In addition, this lab lacked a tool for precise extraction of dead embryos and waste product, which may have led to live embryos being removed accidentally. This also made it difficult to get an equal number of embryos into each well. Ideally, this lab would have spanned a greater number of days to observe how the ethanol impacted the overall life span, eating, swimming and reproduction. In addition, this lab would have employed the use of a micropipette, had a greater sample size, and would have performed a post-mortem dissection to observe the effects of ethanol on the vital organs.

References

Basics about FASDs | CDC. (2018, May 10). Retrieved February 26, 2019, from https://www.cdc.gov/ncbddd/fasd/facts.html

Burke, E. (2016, October 14). Why Use Zebrafish to Study Human Diseases? Retrieved February 26, 2019, from

Petering, D. H., Berg, C., Tomasiewicz, H., Carvan, M., Hesselbach, R., Petering, L. (2018). Zebrafish as Models: Studying the Effects of Environmental Agents on Human Health, retrieved from https://guides.library.uwm.edu/ld.php?content_id=2010971