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The Effects of Caffeine Exposure at Different Rates on Development and Mortality Rates of Zebrafish Embryos

Abstract

Caffeine is one of the most widely used drugs, roughly 90 percent of Americans consume it daily. However, it is often not seen as a drug because it is so widely used. In this experiment 56 zebrafish embryos were exposed to 0.75 mg/mL 9 hours post fertilization and 48 hours post fertilization. Sixty zebrafish were used as control and were not exposed to the drug. The developing embryos were inspected at 48, 72, and 96 hours postfertilization (hpf) to determine deformities, deaths, and to change solutions. The embryos were a representation of developing fetus in a pregnant woman and were used because caffeine can be hazardous to pregnant women. It was found that zebrafish exposed to caffeine immediately had more deformities and death than zebrafish exposed to caffeine 48 hours later. A t test determined a significance in data between the control group and each experimental group. Caffeine is commonly consumed by a huge population without a thought on the potential health risks. These findings can help people watch their intake of caffeine and reduce caffeine intake at young ages.

Introduction

About 90 percent of Americans consume caffeine every day. Half of those people consume 300mg per day, where 400mg is the suggested limit of caffeine before it will cause serious harm to your body. This makes caffeine America's most popular drug. Caffeine is an addictive drug that binds to the adenosine receptors and increases neuron firing. Jitteriness, anxiety, heart problems, and alertness are all effects of this. Withdrawal of caffeine can cause headaches and drowsiness (About Caffeine,2017).

Zebrafish are a small freshwater fish with a high fecundity. Zebrafish embryos grow rapidly and most organs are developed three days after fertilization. Zebrafish are easy to monitor because they are transparent so scientists are able to see the development of the organs with little technology.(Rerio, 2014). The zebrafish share 70 percent of their genes with humans. Zebrafish are also very cheap to buy in comparison to other lab animals (Burke, 2016).

How will caffeine exposure to developing zebrafish embryos affect the survival rate of embryos? It is hypothesised that caffeine exposure to developing zebrafish will cause deformities or death in embryos.

Materials and Methods

<u>Materials</u>

- Clean sanitized plate
- 96 zebrafish embryos
- Tape and marker for labeling
- Instant Ocean solution (for control)
- 0.75 mg/mL caffeine solution
- Dissecting microscope
- Incubator (28.5 degrees Celsius)
- Waste container
- 1 mL pipettes
- Methane blue (prevent bacteria growth)

Procedure

Day 1

1. Obtain rinsed embryos from your teacher.
2. Label the plate with the name and class hour. Label the caffeine concentration as well using the sharpie provided.
3. Divide the embryos so there are approximately 10 embryos in each well. Label the plate on the student data sheet. Suck out the old solution. Add appropriate testing solution. This prevents the solutions from being diluted when adding in the embryos.
4. Record exact numbers of live embryos on data sheet.
5. Observe the embryos under the dissecting microscope. Record observations on student data sheet.
6. Place each plate in the 28.5 degree Celsius incubator overnight.

Day 2

1. Remove plate from incubator. Remove dead embryos from plate using the disposable pipette. Squirt dead embryos into waste beaker. Be careful to only remove dead embryos.
2. Count remaining embryos, hatched fish, and record in data table.
3. Remove caffeine solutions from each well of the plate.
4. Replace the caffeine solutions with the appropriate fresh caffeine solution using a clean pipette each time.
5. Place plate under dissecting microscope and record observations on student data sheet. Note/describe any developmental abnormalities and developmental markers. Repeat for all caffeine concentrations.
6. Remove 1-2 embryos and place on the depression slide with cover slip. Observe the embryo using the compressed microscope. Record observations on student data sheet repeat for all caffeine concentrations.
7. Return the embryos to the well in the plate.
8. Return the plate to the 28.5 degree Celsius incubator.

Day 3

1. Repeat Day 2 work and observations. Record all data.
2. If one does not wish to further the experiment, then place all embryos and fish in waste container. The teacher will properly dispose of the organisms.

Day 4

1. Repeat Day 2 work and observations. Record all data.
2. Place embryos and fish in waste container. The teacher will properly dispose of the organisms.

This procedure was created by SEPA UW- Milwaukee.

Safety Precautions

- Wash hands before and after handling zebrafish embryos, this will prevent contamination and consumption of harmful chemicals.
- Wear gloves and safety glasses.

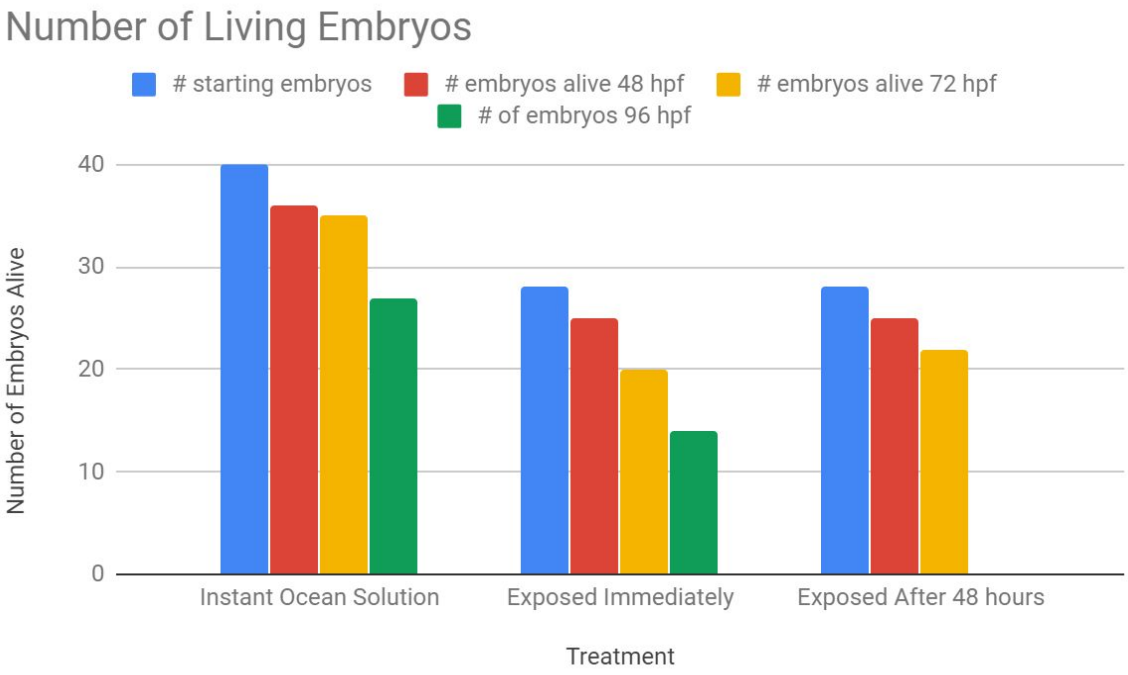
Data

Data Table 1
Embryos alive after exposure to Instant Ocean, 0.75mg/mL caffeine immediately, and 0.75mg/mL after 48 hours at 48 hpf, 72 hpf, and 96 hpf.

Number of Living Embryos

Treatment	# starting embryos	# embryos alive 48 hpf	# embryos alive 72 hpf	# of embryos 96 hpf
Instant Ocean Solution	40	36	35	27
Exposed Immediately	28	25	20	14
Exposed After 48 hours	28	25	22	19

Figure 1 The number of surviving zebrafish at 48 hpf, 72 hpf, and 96 hpf.



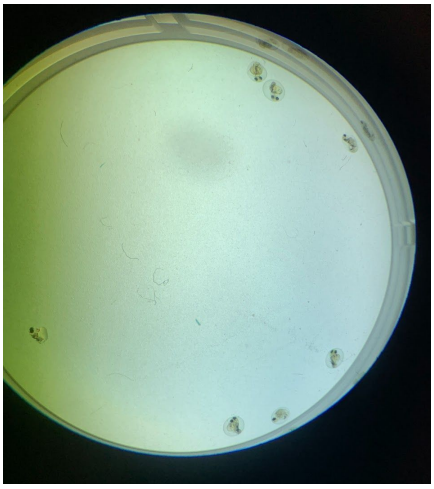


Figure 2
This shows the zebrafish embryos exposed to the caffeine immediately at 72 hpf. No zebrafish this experimental group have hatched.

Figure 3
This shows the zebrafish embryos exposed to caffeine embryos in exposed after 48 hpf at 72 hpf. all zebrafish embryos in this experimental group have hatched.

Data Table 2 - Significance of Data In Comparison WIth the Other Sets of Data at 96 hpf.

T test results comparing each experimental group to the control and comparing the experimental groups to each other.

Data Being Compared	T Test Result	Significance
Control vs. Exposed Immediately	0.0231	Yes
Control vs. Exposed After 48 Hours	0.0283	Yes
Exposed Immediately vs Exposed After 48 Hours	0.2534	No

T tests were used because it compares averages of two sets of data to determine if a variable caused a scientific significant change of the data rather than a random chance.

Results

In this experiment each well of the control was filled with ten zebrafish embryos and exposed to the Instant Ocean solution. The wells exposed to the caffeine were filled with seven zebrafish embryos. Some of the experimental wells were exposed to caffeine nine hours post fertilization while the others were exposed to caffeine 48 hours post fertilization. One concentration of caffeine was used. The independent variable was the timing of the exposure to caffeine. The dependent variable was the death rate and deformities of the zebrafish embryos. The control were wells exposed to only the Instant Ocean solution. The results showed that there was a slight impact on the zebrafish embryos relating to the timing of their exposure to caffeine. The sooner the zebrafish were exposed to caffeine the lower the chances of the zebrafish embryos hatching.

Discussion

In this experiment, the question being tested was if caffeine exposure at differing times during development can have an effect on the development and mortality rates of the zebrafish embryos. The data linked a slight difference in mortality rates with zebrafish that were exposed to the caffeine after fewer hours. This supports the hypothesis because the zebrafish that were exposed to the caffeine immediately had a higher mortality rate than the zebrafish exposed to the caffeine after 48 hours. Figure 2 and Figure 3 show the zebrafish that were exposed to the caffeine immediately did not hatch from the eggs whereas all of the alive zebrafish exposed to the caffeine after 48 hours hatched. The T test results of the mortality rates reported that there was not a significance of the data between the zebrafish exposed immediately and the zebrafish exposed after 48 hours, however there was a significance of data from the t test result between the control group and both sets of experimental fish. An error or limitation of this experiment was the lack of class time and death of many zebrafish embryos in transport to the school. Another error was the potential to miscount the zebrafish or accidentally suck up zebrafish while changing the solutions in the wells. It was found that many of the surviving zebrafish had not hatched after 96 hours.

References

About Caffeine. (n.d.). Retrieved October 19, 2017, from https://www1.villanova.edu/villanova/studentlife/health/promotion/goto/resources/drugs/caffeine.html

Burke, E (2016, August 9). Why Use Zebrafish to Study Human Diseases? (2016, October 14). Retrieved November 21, 2017, from https://irp.nih.gov/blog/post/2016/08/why-use-zebrafish-to-study-human-diseases

Distelberg, B. J. (2017, June 1). The Effects of Coffee and Caffeine on Mood, Sleep, and Health Related Quality of Life. Retrieved November 17, 2017, from http://online.liebertpub.com/doi/abs/10.1089/jcr.2016.0023

James, J. E. (n.d.). Dietary Caffeine and Pregnancy Outcomes . Retrieved November 20, 2017, from http://online.liebertpub.com/doi/full/10.1089/jcr.2013.1229

Petering, David H., Craig Berg, Henry Tomasiewicz, Michael Carvan, Louise Petering, and Renee Hesselbach. "Zebrafish as Models: Studying the Effects of Environmental Agents on Human Health." 1
-96.

Rana, N. (2010, April 26). Zebrafish. Retrieved November 17, 2017, from http://online.liebertpub.com/doi/abs/10.1089/zeb.2009.0631

Why use the zebrafish in research? (2014, November 17). Retrieved October 23, 2017, from https://www.yourgenome.org/facts/why-use-the-zebrafish-in-research

Yekti, A.P.A., Hsu H., Wang W. (2014, June 1). The Effect of Paclobutrazol on the Development of Zebrafish (Danio Rerio) Embryos Retrieved November 21, 2017