The Effect of Caffeine concentration on Zebrafish Embryo Development

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Abstract

This lab was conducted to test the effect of differing caffeine concentrations on the development of zebrafish embryos. The outcome of this lab supported the hypothesis that if zebrafish are exposed to higher concentrations of caffeine, then they are more likely to have curvature in the spine. Research has shown that caffeine exposure during development impacts brain function and stunts growth. (Healthline, 2017)

In this lab 120 Zebrafish embryos were exposed to two different concentrations of caffeine: 0.25mg/mL and 0.50mg/mL. The solutions were replaced every day and observed under a microscope. After four days, the number of zebrafish with any curvature in the spine were recorded. After four days it was recorded that 33.67 percent of zebrafish exposed to 0.25mg/mL of caffeine had a curved spine and 55.53 percent of zebrafish exposed to 0.5mg/mL of caffeine had a curved spine. Between both concentrations of caffeine there was an 18.66 percent increase in the average percent of zebrafish with a curved spine, which shows that with higher concentrations of caffeine, the greater the chance of development issues. The P-value of 0.0027 was also very statistically significant.

Background Information

Why Fish?

Model organisms are non human species that scientists can use to help them better understand more about biology and how it works. Zebrafish are a good model for development because many generations can be studied at once, and they can create detailed genetic maps (YourGenome, 2014). Zebrafish are also a good model for human development because they share 70 percent of genes with humans and they have many of the same vital organs and tissues that humans have (YourGenome, 2014). Humans and Zebrafish are very similar, so testing variables on zebrafish can give us a more accurate representation of what will happen to humans under a similar condition.

Caffeine

The zebrafish model can tell us whether or not caffeine has a negative effect on development. Research has shown that excessive caffeine intake during development results in complications in health, such as stunted growth and negatively impacts the function of the brain (Healthline, 2017).

In fact, a study conducted by The Swiss National Science Foundation (SNSF) showed a slower maturing rate for the brains of rats exposed to caffeine during development. In this study 30-day old rats were given a moderate amount of caffeine over a span of five days. The result was a delayed maturing process in the brain (Science Daily, 2013).

This zebrafish model lab also showed that high concentrations of caffeine led to a highly increased chance of spine deformities. Research and the zebrafish model suggests that in order to

fully grow and develop, caffeine should not be present in the critical developing stages of an organism's life. (US National Library of Medicine, 2009)

The Investigation

In this lab, 120 zebrafish embryos were exposed to two different concentrations of caffeine, 0.5mg/mL and 0.25mg/mL and observed under a microscope over four days. This lab occurred to test the hypothesis that if zebrafish are exposed to higher concentrations of caffeine, then they are more likely to have curvature in the spine.

Materials and Methods

Participants

In honors biology, November 2019, students at greendale high school studied the effect of different chemicals on the development of zebrafish embryos. Over three days, the zebrafish embryos from Milwaukee School of FreshWater Sciences, were exposed to different concentrations of chosen chemicals and were observed by the students.

Materials

- 12 welled falcon plate
- 120 zebrafish embryos
- 1 beaker for waste
- disposable pipettes
- Incubator for fish embryos
- 1 compound microscope
- Stock solution for caffeine (0.25, 0.5 mg/mL)
- Instant ocean solution (control)

Design

The variable that is being measured in this lab is the variety of physical defects that caffeine has on Zebrafish embryos. The independent variable in this lab was the concentration of caffeine (0.25 and 0.5 mg/ml) and the dependent variable was the amount of fish that had physical defects. The constants are the starting amount of embryos in each well forty control and forty experimental (20 in each experimental group), and the amount of solution in each well.

Procedure

Day one

1. Place 10 zebrafish embryos in each well of the 12 welled falcon dish using a disposable pipette
2. Remove any dead embryos using a disposable pipette and put in waste beaker
3. Observe using a microscope
4. Place 12 welled falcon dish in incubator

Day 2

5. Replace the control (instant ocean) in wells: A1, B1, and C1 with 0.5 mg/ml concentration of caffeine using a disposable pipette
6. Replace the control (instant ocean) in wells: A2, B2, and C2 with 0.25 mg/ml concentration of caffeine using a disposable pipette
7. Replace the control (instant ocean) with more instant ocean in wells: A3, B3, C3, A4, B4, and C4 using a disposable pipette
8. Observe using microscope
9. Place 12 welled falcon dish in incubator

Day 3

10. Replace the control (instant ocean) in wells: A1, B1, and C1 with 0.5 mg/ml concentration of caffeine using a disposable pipette
11. Replace the control (instant ocean) in wells: A2, B2, and C2 with 0.25 mg/ml concentration of caffeine using a disposable pipette
12. Replace the control (instant ocean) with more instant ocean in wells: A3, B3, C3, A4, B4, and C4 using a disposable pipette
13. Observe using microscope
14. Place 12 welled falcon dish in incubator

Day 4

15. Observe all zebrafish embryos with a microscope

16. Make note of any zebrafish embryos with physical defects

(University of Wisconsin, n.d.)

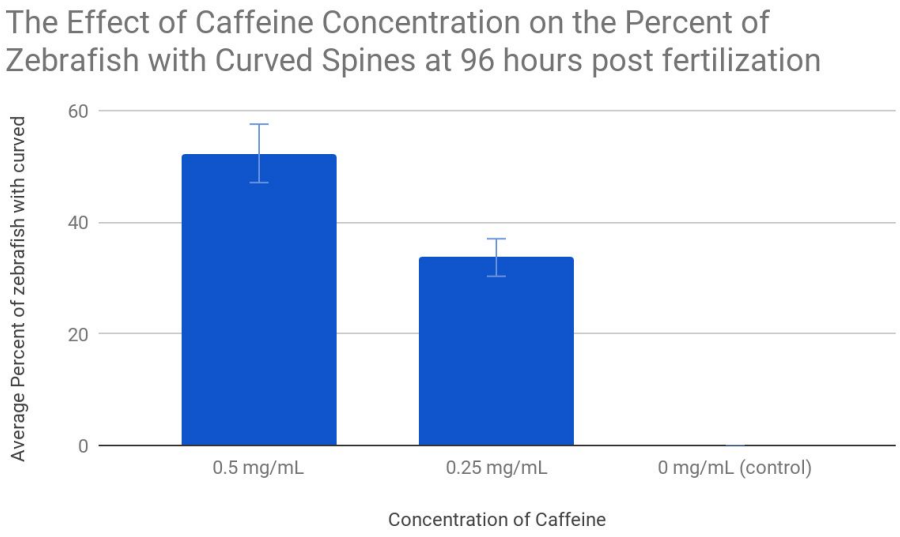
Results

Summary of Results

120 Zebrafish embryos were separated evenly into a 12 welled falcon dish. They were then exposed to the concentrations of caffeine: 0.5mg/mL, 0.25mg/mL, and instant ocean (control). The amount of zebrafish with curved spine were recorded after 96 hours. It was observed that as the concentration of caffeine increased, the number of Zebrafish with a curved spine increased.

Tables and Figures

Graph 1:



Graph 1: shows the effect of differing caffeine concentrations on the average percent of zebrafish with a curved spine

The Effect of caffeine Concentration on the Percent of Zebrafish with Curved Spines at 96 Hours Post Fertilization

Table 1:

	0.5 mg/mL	0.25 mg/mL	0 mg/mL (control)
Average Percent of zebrafish with curved spine	52.33	33.67	0

Table 1: Shows the average percent of zebrafish with a curved spine.This data is represented in graph 1

Image 1:

Image 1: Two zebrafish exposed to 0.25 mg/mL concentration of caffeine

Image 2:

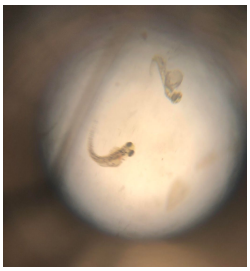


Image 2: Two zebrafish exposed to 0.5 mg/mL concentration of caffeine

Statistical Findings

Table 2:

The Average Percent of Zebrafish with a Curved Spine

	control	0.5 mg/mL caffeine	0.25 mg/mL caffeine
Mean	0	52.33	33.67
Median	0	50	17
Mode	0	none	17
Sample size	22	15	15

P-value: 0.0027

Table 2: Shows the descriptive statics of the average percent of zebrafish with a curved spine

The mean, median, and mode (descriptive statistics) of the average percent of Zebrafish with a curved spine, all increased as the concentration of caffeine increased. Between the two concentrations of 0.25 mg/mL and 0.5 mg/mL, there was an 18.66 percent increase in average zebrafish with a curved spine. The results of this lab were significant because they show that the higher the concentration of caffeine, the higher the average amount of zebrafish with a curved spine is. The P-value is 0.0027 which also suggests that the results of this lab are very statistically significant.

Discussion

Importance of the Topic

The purpose of this lab was to show the effect of caffeine on the average percent of zebrafish with a curved spine to understand the effect of caffeine on development. Studies have shown that caffeine affects brain development and stunted growth due to the exposure of caffeine during development. The results of this lab supported the idea that caffeine should not be present during the vital stages of development.

Importance of the Findings

The findings in this lab significantly supported the hypothesis that if zebrafish are exposed to higher concentrations of caffeine, then they are more likely to have curvature in the spine. Zebrafish embryos exposed to two different concentrations of caffeine experienced a curved spine. When exposed to 0.5mg/mL of caffeine 52.33 percent of zebrafish were observed to have a curved spine, while in 0.25mg/mL only 33.67 percent of zebrafish had a curved spine. This shows that the higher the concentration of caffeine a developing embryo is exposed to, the greater the chance of it having a physical defect. The p-value of 0.0027 also demonstrated that the findings in this lab were very statistically significant and supported the fact that caffeine negatively affects development.

There were a few limitations to this lab along with minor errors. One limitation was that there was a limited amount of time, which was four 50 minute class periods. There was also the limitation of not being able to observe the fish throughout the day, with the limited time periods the embryos were only able to be observed once a day. One error happened in this lab in which some embryos were accidentally removed from wells during the process of switching solutions everyday. So even though most wells began with 10 embryos, many ended up with only six, but the lab still produced results that statistically supported the hypothesis.

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