

Abstract

Although the use of cigarettes has decreased dramatically, around 10% of women during pregnancy still expose their developing child to nicotine, whether that be by smoking cigarettes, e-cigarettes, or using nicotine patches. Some research done at Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire have suggest that the use of nicotine during pregnancy can increase the risk of sudden infant death syndrome, which is the sudden and unexpected death of an infant under 12 months of age that occurs typically while sleeping. (Physiologically Study, 2018) This study investigates the effect of nicotine on developing zebrafish embryos, which can help researchers understand how nicotine can affect a human embryo during development if they are somehow exposed to it. Overall, this experiment showed that zebrafish that were exposed to nicotine solution had physical differences when in comparison to normal zebrafish, but the data of this study turned out to not be significant.

Materials and Methods

- 4x3 well plate
- A dissecting microscope
- Large and minimum bore transfer pipettes
- A large beaker
- Tape
- An incubator set at 28.5°C
- Bottle of 0.2 mg/mL nicotine solution
- Bottle of 0.05 mg/mL nicotine solution
- Instant Ocean/Embryo Media Solution
- A depression slide with cover slip

Day 1: Embryos were obtained from teacher and side of a 4x3 well plate was labeled. Wells in row A were then filled ¾ of the way with Ocean/Embryo Media solution. Row B of the well were filled with 0.05 mg/mL nicotine solution, and row C were filled with 0.2 mg/mL nicotine solution. 5-10 embryos were put into each well. Embryos were observed under a dissecting microscope and data was recorded. Each plate was placed in incubator overnight.

Day 2, 3: Plates were removed from the incubator. All dead embryos were disposed of. Remaining embryos and the hatched fishes in each plate were recorded. Nicotine solution was replaced with appropriate fresh nicotine solution. Plate was observed under a microscope and data was recorded. Embryos were placed back into the incubator.

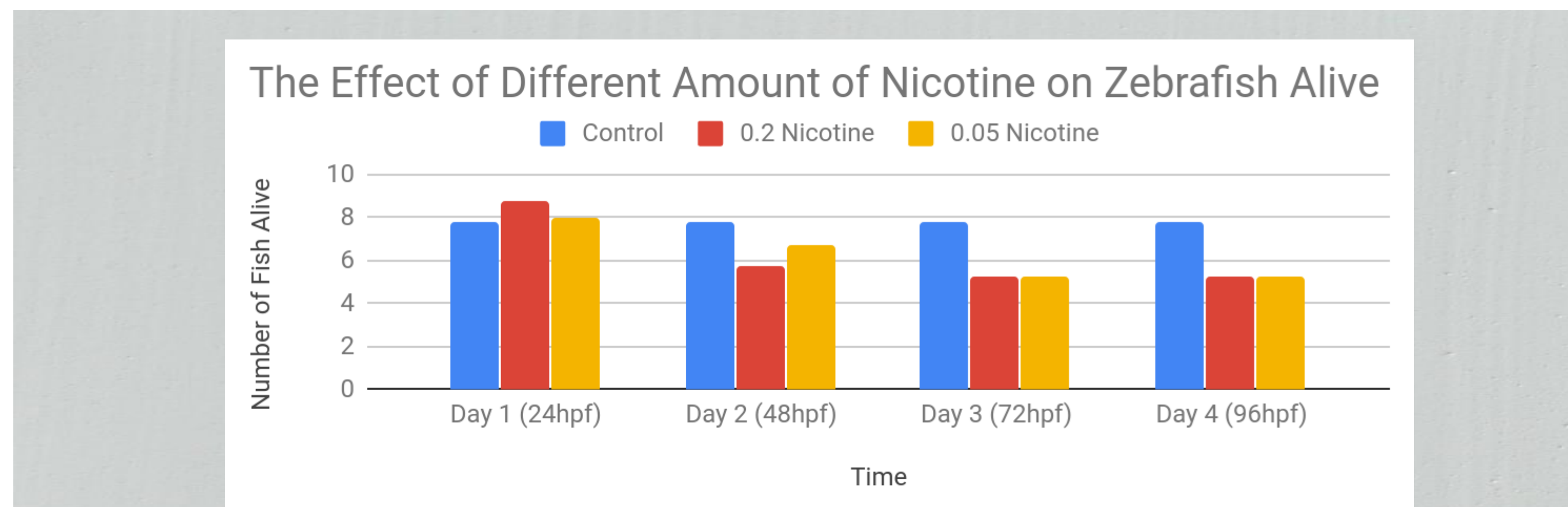
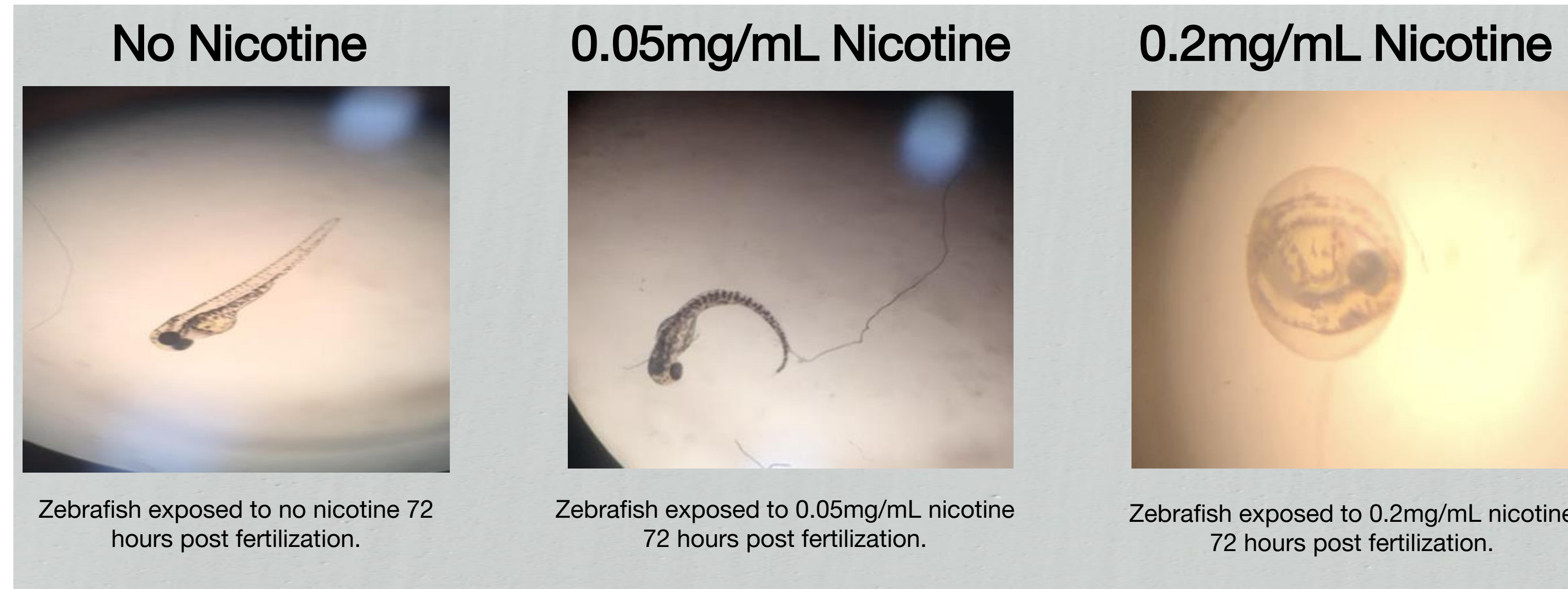
Day 4: Same procedure was used as in previous days. All data were recorded. All the fish and embryos were placed in the waste beaker so that it can properly be disposed of.

- Control Group: Non-nicotine exposed fish
- Independent Variable: Concentration of Nicotine fish were exposed to
- Dependent Variable: # of zebrafish dead, and physical abnormalities

Introduction

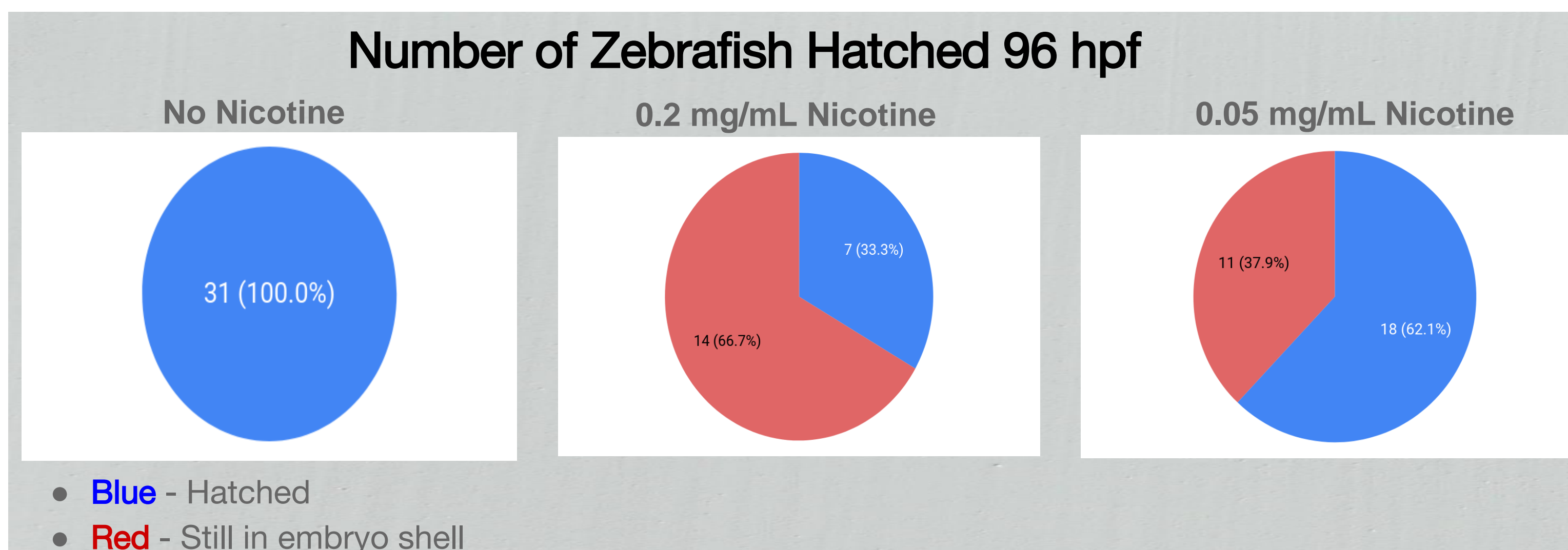
When a person exposes themselves to nicotine, the nicotine stimulates the body and causes an increase in blood pressure, respiration, and heart rate, it activates reward pathways in the brain that regulates reinforcement and feelings of pleasure. (National Institute of Drug Abuse, 2018). Nicotine have been known to cause many negative effects of a human body. There have been studies done that shows it can have even a bigger effect of embryos. There have been a correlation between the use of nicotine and increased birth defects and sudden infant death syndrome, but no definite studies have been able to prove a direct causation. In this experiment, Zebrafish were used to stimulate the effect that nicotine may have on a developing embryo, Zebrafish share about 70% of genes with humans, so modeling a disease or birth defect in the fish can easily be done (Burke, 2016). Many of the critical organs found in humans are also found in Zebrafish, making Zebrafish some of the best models (Burke, 2016). Zebrafish were used for the model organism in this experiment because the fish clearly showed the similar development between the fish and a baby in the womb.

Hypothesis: If the concentration of nicotine in the solutions were higher, than there would be more zebrafishes affected and/or dead.



Please note that in the graph above:

- “alive” refers to the number of zebrafish that were still in their egg *and* zebrafish that were hatched
- This is the average of each well. It is the number of zebrafish that were still alive each day
- Fish were determined to be alive after they were stimulated w/ a pipette and if they had moved / reacted



Results

Overall, the nicotine showed a significant effect on the development of the zebrafish embryos In this experiment, the data concluded that:

- More fishes died in 0.2mg/mL nicotine solution than in the 0.05 mg/mL and normal solution.
- As the concentration of nicotine went higher, more zebrafish did not hatch.
- Left in nicotine solution, the zebrafish became deformed (curved spines).
- Zebrafish that that were exposed to nicotine did not have as strong of a response to stimuli as the control group of zebrafish.

However, when data was entered into GraphPad, a software program used to analyze scientific data, the p-value turned out to be 0.2504. This showed that our data was not significant.

Discussion

The hypothesis that this experiment was based off was that if the concentration of nicotine in the solutions were higher, than there would be more zebrafishes affected and/or dead. The results from this experiment support part the hypothesis, but it was not conclusive. Qualitative data gathered throughout this experiment shows that the nicotine clearly had an effect on the fish, as the fishes not exposed to nicotine solution had straight spines and fishes that were exposed to nicotine solution had curved spines. This part of the study may help show the effect that nicotine may have on developing human embryos because of the way humans and zebrafish develop so similarly. But, collected qualitative data did not support the hypothesis that if the concentration was higher, than more zebrafish would be affect. The zebrafishes exposed to the nicotine, regardless of concentration, showed the same affect. Quantitative data show that this experiment was not significant. However, there was a decrease in alive and hatched zebrafishes that were placed in nicotine solution. Although this is only a correlation, it may help in future research on the effect that nicotine on developing embryo eggs.

One source of error that occurred that might have affected the significance of this experiment was that there were less fish that appeared on later days of the experiment than in earlier days. This could’ve been because some fishes were accidentally sucked up when the wells were refilled with fresh nicotine solution. It could also be because of the number of zebrafish that were selected during the beginning of the experiment, as the number of zebrafish selected were an arbitrary number between 5 - 10. This could’ve affected our p-value

In conclusion, this experiment showed that while nicotine may have an effect on developing embryo, the data collected during this experiment are not conclusive enough to show that higher amounts of nicotine concentration had more of an effect on the developing embryos.

Reference

The Physiological Society. (2018). Use of nicotine during pregnancy may increase risk of sudden infant death syndrome. Retrieved from <https://www.sciencedaily.com/releases/2018/07/180718223057.htm>

WInSTEP Environmental Health Science Program: Zebrafish. (n.d.). Retrieved from <https://guides.library.uwm.edu/c.php?g=56496&p=364360>

Burke, E. (2016, August 9). *Why Use Zebrafish to Study Human Diseases?* Retrieved from irp.nih.gov.

National Institute on Drug Abuse. (n.d.). How does tobacco deliver its effects? Retrieved from <https://www.drugabuse.gov/publications/research-reports/tobacco-nicotine-e-cigarettes/how-does-tobacco-deliver-its-effects>