# The Embryonic Development, Survival, and Hatched Rate of Zebrafish When Exposed to Caffeine Over 72 Hours

*Rebecca Hunter & Ian Vang*
*Waukesha North High School*

## Abstract

Caffeine is a drug that can be found in items such as coffee, tea, soda, chocolate, some energy drinks, and medicines. Research has found that caffeine can cause birth defects such as an increase in fetal heart rate, a decrease in growth rate, and a high risk of miscarriage. It's important to know the effects on zebrafish embryos, so the data can be analyzed to find how the zebrafish have been affected, and how they were affected. Humans and zebrafish are similar and have similar DNA meaning they react in close to the same way. Zebrafish can help people understand the health risks that can come with the intake of caffeine. The reason behind this experiment was to observe the survival rate of zebrafish embryos when developing in concentrations of caffeine. Over 4 days, a well plate was used to contain the zebrafish in increasing concentrations of caffeine (0.0 mg/mL, 0.05 mg/mL, 0.25 mg/mL, & 1.0 mg/mL). During the experiment, the focus was to collect data on the mortality and development rate of the zebrafish when in different concentrations of caffeine. The results show, the number of zebrafish in higher concentrations of caffeine would decrease and would hatch quicker than lower concentrations. This data was used to calculate a chi-square analysis to find if the results were by chance or if the caffeine truly affected the embryos. This would mean that the data was not by chance and that the zebrafish were affected by the caffeine. The experiment was conducted on zebrafish embryos to compare how caffeine might affect humans. The results show that if high amounts of caffeine were to be consumed by someone pregnant, it could lead to the fetus having a faster heart rate and possible miscarriage.

# Introduction

Caffeine is a stimulant found in many drinks and some medicine. The purpose of this experiment was to find the effects of caffeine on zebrafish embryos and relate the results to embryonic development. The experiment was conducted to identify how many zebrafish were alive and hatched as the concentration of caffeine increased. According to Caffeine In Pregnancy (2015), “Caffeine slightly increases your blood pressure and heart rate and the amount of urine your body makes. Caffeine may cause you to feel jittery, have indigestion or have trouble sleeping.” This would indicate that the increased intake of caffeine would cause the body to have a higher heart rate, indicating that the increase of the zebrafish’s heartbeat will be harder and faster, lowering the survival rate. Caffeine has also shown to raise the risk of miscarriages in some studies Searing (2012), “research has shown that it can increase the baby's heartbeat, particularly in the first trimester.” Doctors and researchers recommended reducing an expecting mother’s intake of caffeine to lessen the risk of a miscarriage. Other problems can occur when too much caffeine is ingested while pregnant, Riccardi (2004), “Also, consuming more than 300 milligrams of caffeine a day (the amount in two 5-ounce brewed cups) increases the risk of miscarriage, fetal growth problems and low birth weight.” Zebrafish would have a similar reaction to caffeine because they are more closely related to humans than other commonly used invertebrates since they are more similar they are most likely to have features that are close to humans. The research done with zebrafish can be related to humans and can help people understand the effect of caffeine on humans. It was hypothesized that if the zebrafish embryos are exposed to increasing amounts of caffeine, it will then cause fewer embryos to survive and hatch than the control group of embryos that have 0.0 mg/mL caffeine solution because studies have shown in pregnancies that too much caffeine can cause a miscarriage.

# Materials and Methods

The materials used consist of stock solutions of caffeine (0.0, 0.05, 0.25, & 1.0 mg/mL caffeine) that was contained in 100 mL beakers, 50 mL beakers for dead embryos and disposal of old liquid, a sharpie, instant ocean/embryo media solution, and two types of disposable pipettes (one large-bore & one fine bore). In addition, painters tape was

used, a well plate to house all of the embryos that are developing in a concentrated solution, an incubator that is set at 28.5 °C, dissecting microscope, and 40 embryos. For safety, gloves were worn when working with the solutions.

Day 1: As the experiment was conducted, these steps were followed. The first day rinsed embryos were obtained from the teacher and four of the wells from a well plate were used. A well plate lid was labeled using painters tape that had solution concentration, students names, and class hour on it. Afterward, ten zebrafish embryos were put in each well using a large-bore disposable pipette. To keep the experiment as accurate as possible, residual liquid from the embryos transfer was removed using a fine bore disposable pipette. Next, 1 mL of each concentration was put into the wells. The first well had a 0.0 mg/mL concentration of caffeine, the second had a 0.05 mg/mL concentration, the third had a 0.25 mg/mL concentration, and the fourth had a 1.0 mg/mL concentration. The embryos were then observed under a dissecting microscope. Pictures and initial observations were taken, a recount of the embryos was done to ensure none were lost and all were alive. The embryos were placed in an incubator heating at 28.5 °C overnight for 24 hours.

Day 2-3: The next day the well plate was removed from the incubator, dead embryos, and old solutions were removed from the wells and emptied into a waste beaker. The caffeine concentrations were removed and replaced with fresh solution. Remaining embryos were counted and it was observed how many had hatched and how many were alive, the data was recorded in a data table. It was observed whether the Zebrafish embryos were dead or alive by observing the embryos through a microscope and checking if the zebrafish had a heart, eyes, and were moving. Dead embryos had turned into a cloudy white color, and some could be seen with protozoa when looking under a microscope eating away at the dead embryos. Pictures and observations were then taken.

Day 4: Data was recorded and pictures were taken. The surviving embryos/living fish were placed in a tank. A chi-square analysis was completed on the data to ensure statistical significance.

# Results

The purpose of the experiment was to identify the effect of caffeine on zebrafish embryos to overall see how it would affect fetal development. The lab was set up with

increasing concentrations of caffeine to easily see the impact of caffeine on the mortality rate and the hatch rate of zebrafish embryos.

Table 1: Raw Data Table of Hatched and Alive Embryos.
The raw data from the experiment showing the number of hatched and alive embryos over 72 hours.

| *Treatment* | *# of starting fish* | *24 hours post fertilization* | | *48 hours post fertilization* | | *72 hours post fertilization* | |
|---|---|---|---|---|---|---|---|
| Caffeine | 10 | # Hatched | # Live | # Hatched | # Live | # Hatched | # Live |
| 0.0 mg/mL *(Control)* | 10 | 0 | 9 | 2 | 9 | 9 | 9 |
| 0.05 mg/mL | 10 | 0 | 10 | 4 | 10 | 10 | 10 |
| 0.25 mg/mL | 10 | 0 | 9 | 6 | 1 | 7 | 1 |
| 1.0 mg/mL | 10 | 0 | 10 | 0 | 5 | 5 | 0 |

The dependent variable of the experiment was the mortality rate and hatch rate of zebrafish embryos. The independent variables in the experiment were 0.0 mg/mL of caffeine, 0.05 mg/mL of caffeine, 0.25 mg/mL of caffeine, and 1.0 mg/mL of caffeine. The control group was 0.0 mg/mL of caffeine (Table 1). The controlled variables were

the same amount of solution in each well, the temperature of the incubator (28.5 °C) and the time spent in it, the size of the wells, and the amount of zebrafish in each well.

A chi-square analysis was done to see if the result occurred on accident or if the zebrafish were truly affected by the caffeine solutions. The chi-square value was 32.8, and the null hypothesis was rejected using a degree of freedom of 3, and a critical value of 7.82. This means that the results were not by chance, and the caffeine solutions did have an effect on the zebrafish because the chi-square value (32.8) was higher than the critical value.

The data showed over time that as the concentration of the solution increased, more embryos died (figure 1). Embryos in 0.25 mg/mL and 1.0 mg/mL caffeine concentrations had all died, or only one survived, but most likely didn't survive much longer. Fewer embryos seemed to hatch before dying when in the higher concentrations (figure 2), such as 0.25 mg/mL and 1.0 mg/mL. In the 1.0 mg/mL caffeine solution, only five embryos hatched but then died at some point in the incubator before data was collected.

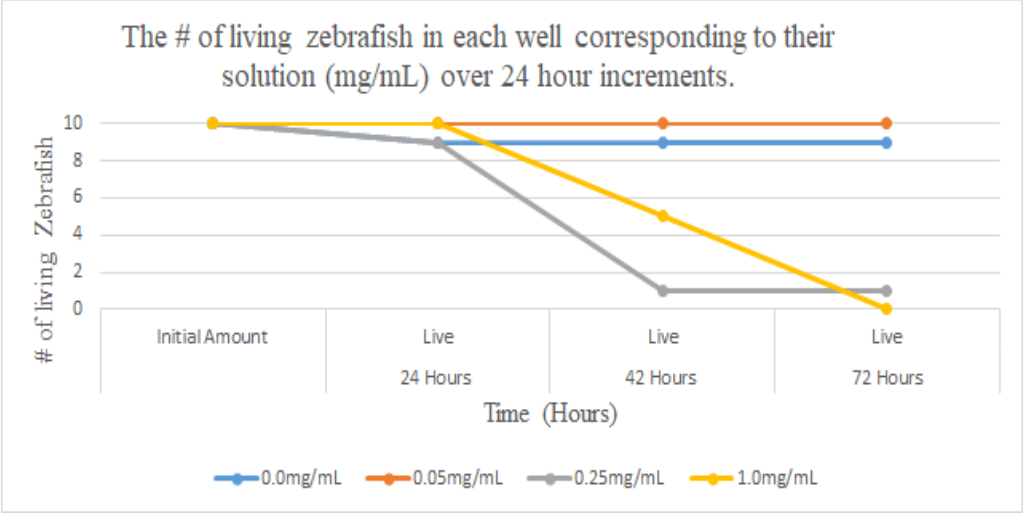


Figure 1: The Mortality of Zebrafish Embryos When Exposed to Caffeine
The graph shows the relationship between the solution concentration and how many embryos are alive. The higher solution concentrations have less embryos after 72 hours.

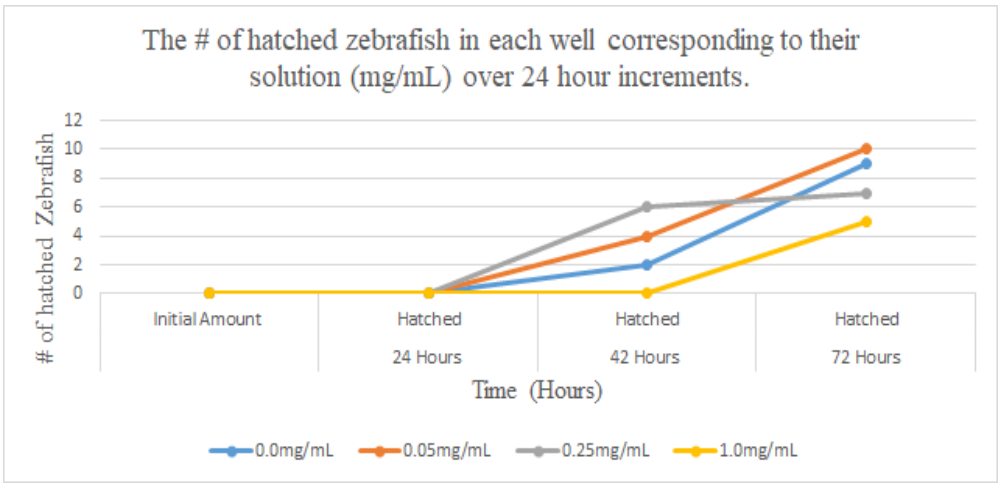


Figure 2: The Effect of Caffeine on the Rate of Hatching
The graph shows the relationship between the number of hatched embryos and solution concentrations.

Embryos in the 0.05 mg/mL solution all hatched and survived.

# Discussion

This experiment proved the hypothesis if the zebrafish embryos are exposed to increasing amounts of caffeine, it will then cause fewer embryos to survive and hatch than the control group of embryos that have 0.0 mg/mL caffeine solution because studies have shown in pregnancies that too much caffeine can cause a miscarriage. The data in the experiment relates to the research which showed that caffeine can cause miscarriages and other issues while being pregnant. As stated earlier, Riccardi stated that consuming over 300 milligrams of caffeine a day can result in birth defects such as miscarriage, birth weight, and fetal growth. This was supported in the experiment because as the concentrations of caffeine increased, the number of living embryos would decrease. Also previously stated by Linda Searing, research has shown that caffeine can increase a baby's heartbeat, particularly in the first trimester. The results from the experiment support the hypothesis to be correct because the higher the concentration, the fewer living embryos would be present. Errors that were encountered consisted of embryos dying before recording data on them and mixing concentrated solutions. For this experiment to be as accurate as possible, it would be advised to repeat the process multiple times and have a larger sample size to ensure the results stay very similar to one another. Some other ways that could be done would be to add more embryos in each well. The data showed that 0.25 mg/mL did have an impact and killed most of the embryos. The 0.05 mg/mL caffeine solution did not impact the zebrafish embryos. A way to resolve this would be to have more diluted solutions of caffeine, and/or have another well plate in the experiment. This could lead to research that can be done to find the limit of caffeine someone could have while pregnant without harming the baby.

# References and Literature Cited

Bayer, B.M. (2019). Caffeine. In *World Book Student*. Retrieved from https://www.worldbookonline.com/student-new/#/article/home/ar086900

Caffeine in pregnancy. (n.d.). Retrieved November 7, 2019, from https://www.marchofdimes.org/pregnancy/caffeine-in-pregnancy.aspx

Caffeine. (2019). In *Encyclopædia Britannica*. Retrieved from https://school.eb.com/levels/high/article/caffeine/18535

Chen, Y.-H., Huang, Y.-H., Wen, C.-C., Wang, Y.-H., Chen, W.-L., Chen, L.-C., & Tsay, H.-J. (2008). Movement disorder and neuromuscular change in zebrafish embryos after exposure to caffeine. Neurotoxicology and Teratology, 30(5), 440–447. https://doi.org/10.1016/j.ntt.2008.04.003

Riccardi, V. A. (2004). food facts. *Fit Pregnancy*, *10*(6), 98–101. Retrieved from https://search.ebscohost.com/login.aspx?direct=true&AuthType=cookie,ip,cpid&custid=s6269028&db=hxh&AN=12052156&site=ehost-live&scope=site

Searing, Linda (7AD, Winter 2012). Caffeine during pregnancy shows no effect on child behavior in study Caffeine during pregnancy shows no effect on child behavior in study. *Washington Post, The*. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&AuthType=cookie,ip,cpid&custid=s6269028&db=n5h&AN=wapo.6df8f9c2-6a21-11e1-acc6-32fefc7ccd67&site=src_ic-live&scope=site

UnderwoodAug. 7, E., 2013, & Pm, 2:00. (2013, August 7). No coffee for pregnant moms? Retrieved November 7, 2019, from Science | AAAS website: https://www.sciencemag.org/news/2013/08/no-coffee-pregnant-moms

Zebrafish as a developmental system. (n.d.). Retrieved November 10, 2019, from http://people.ucalgary.ca/~browder/virtualembryo/why_fish.html

Zebrafish faqs. (n.d.). Retrieved December 3, 2019, from http://www.uoneuro.uoregon.edu/k12/FAQs.html#Research

Zebrafish faqs. (n.d.). Retrieved November 10, 2019, from http://www.uoneuro.uoregon.edu/k12/FAQs.html#Percent