The Effect of Caffeine on the Mortality Rates of Zebrafish Embryos

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Abstract

- This experiment was done to see the effects of caffeine on Zebrafish embryos as 1 in 5 pregnancies end in miscarriage (Love, Bhattacharya, Smith, et al., 2010).
- The results from this experiment proved the hypothesis that stated: when zebrafish are exposed to varying levels of caffeine, the zebrafish in the less concentrated amounts of caffeine will have lower mortality rates.
- Zebrafish were placed into three different groups, a control of instant ocean, a group containing 0.05mg/ml caffeine, and a group containing .25mg/ml caffeine.
- Data was taken on the data of the fish every 24 hours, with the control groups being added at the 48 hour marks (David, Berg, Tomasiewicz, et al., 2018).
- In the groups of fish with traces of caffeine in them, the mortality rate was higher than that of the fish with none.
- Because of the fact that zebrafish and humans are so closely related, it can be assumed that an increase in mortality rate in zebrafishes from caffeine is also true in humans.
- This is significant as with this information, people can become more educated and stop drinking caffeine while pregnant, possibly reducing the number of miscarriages.

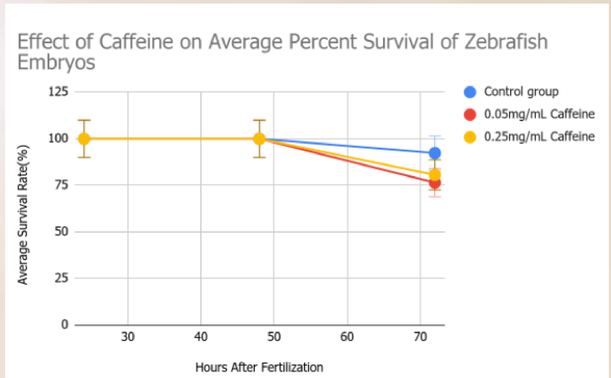
Introduction

- The Purpose of this study was to use different amounts of caffeine on zebrafish embryos to see the effects that they had on them as drinks such as coffees and sodas contain large quantities of caffeine which is often drunk while pregnant.
- As women increase their daily caffeine intake, the risk of miscarriage increases as well (Weng, Odouli, Li, 2008).
- With the data of the mortality rate of the zebrafish based on caffeine, it could be possible to find out the safe amount of caffeine intake during pregnancy and possibly reduce the amount of miscarriages caused by caffeine intake by implementing this data and publishing a safe usage amount.
- To complete this lab, zebrafish were placed into three solutions of caffeine, with there being around 40 zebrafish in each.
- One solution was 0mg/ml caffeine(pure instant ocean), one was 0.05mg/ml caffeine in instant ocean, and another was 0.25mg/ml caffeine in instant ocean.
- The mortality rate of each were then compared to each other in order to come up with data to see how caffeine affects the mortality rate of the fish.
- The hypothesis stated that the higher the amount of caffeine compared to instant ocean there was, the higher the mortality rate of zebrafish there would be.

Materials and Methods

- The control group of the experiment was the zebrafish embryos in the solution of 0mg/ml caffeine, while the variable group was the zebrafish embryos in the solutions of caffeine above 0mg/ml.
- Sample size of the experiment was around 40 embryos per group, with there being 3 groups.
- The constants of this were that all fish were placed in a well with the same amount of fluid with roughly the same amount of fish.
- The number of living embryos after intervals of 24 hours are the outcomes that were measured by counting the living amount
- After each day, or intervals of 24 hours each, data on the number of living, hatched, and dead embryos was recorded.
- This was repeated over several days in order to come up with a conclusion on the data.
- A t-test was then used to compare the different data sets in order to come to a conclusion of whether or not the data had any correlation with the hypothesis that is trying to be proven true.

- Procedure.
- Day 1.
- Label wells on a plate by marking it 1-3 vertically and A-D horizontally so that you have 1A, 1B, 1C, 1D, 2A, ect..
- Place embryos into each well, ensuring that there is around 10 in each.
- Count the amount of living embryos in each well and label it on data sheet.
- Fill each well with 1mL of control unit (Instant Ocean).
- Create a stock solution with a caffeine amount of 0.25mg/ml and a caffeine amount of 0.05mg/ml, with the amount of caffeine being relative to the amount of instant ocean.
- Day 2.
- Receive plate with wells from the teacher.
- Remove dead embryos from wells using the smaller of the two pipettes and place them in the waste beaker.
- Record the remaining amount of embryos and hatched fish on your paper.
- Replace all solutions with fresh solution
- Day 3/4.
- Repeat all steps from day 2.



As the amount of caffeine increases, the mortality rate of zebrafish increases over time, additionally, over time, more fish die.

Effect of Caffeine on Average Percent Survival of Zebrafish Embryos

Hours After Fertilization	Control Group	0.05mg/mL Caffeine	0.25mg/mL Caffeine
24	100	100	100
48	100	100	100
72	92.31	76.47	80.65

As the percentage of caffeine increases, the mortality rate of zebrafish increases over time, additionally, over time, more fish die.

Average Percentage of Survival of Zebrafish Embryos Comparing Control to 5% Caffeine Solution T-test

Test	Results
P-Value	0.5571

Average Percentage of Survival of Zebrafish Embryos Comparing Control to 25% Caffeine Solution T-test

Test	Result
P-Value	0.6054

A slide showing dead zebrafish embryos

Results

- 34 fish were tested in the control solution with no caffeine.
- 34 fish were tested in the 0.05mg/ml caffeine solution, and 35 fish were tested in the 0.25mg/ml solution which were experimental.
- As the amount of caffeine went up, as did the percentage of mortality among the fish.
- The results finished with 92.32% of the control alive, 76.47% of the 0.05mg/ml caffeine solution alive, and 80.65% of the 0.25mg/ml solution alive, which displays the effects of different amounts of caffeine on zebrafish embryos.
- Both the 0.05mg/ml solution and the 0.25mg/ml solution were the experimentals, with them being tested to see the ways in which caffeine affects zebrafish embryos.
- The independent variable was the fluid the zebrafish were in, while the dependant was their mortality rate, showing how with more caffeine, the mortality rate went up and that they are correlated.
- This proves the point of the experiment and shows how mortality rate of the zebrafish and the amount of caffeine they are in are related.

- The results of this experiment showed that after the 72 hour experiment, more zebrafish were alive in the control solution than either of the experimental solutions.
- After performing this experiment, a t-test was performed to see the statistical significance of the experimental solutions to the control group.
- The results of said experiment show that even if more zebrafish were alive in the control group than experimental groups, the data was not significant.
- These results may show an effect of caffeine on zebrafish embryos but additional testing is needed to find out.

Discussion

- When the amount of caffeine in instant ocean is 0mg/ml, the rate at which the zebrafish embryos live is 92.31 percent.
- When the amount of caffeine is 0.05mg/mL, the rate at which the embryos live is 76.47 percent.
- When the amount of caffeine is 0.25mg/mL, the rate at which the embryos live is 80.65 percent.
- This data helps to explain the alternate hypothesis as it shows how when caffeine was added to the instant ocean solution that the zebrafish were placed in their mortality rate increased.
- The data however, when put into a T-test, shows its as being not statistically significant, possibly proving the null hypothesis.
- Trends in the data can be seen with how when caffeine was present in instant ocean, the mortality rate of zebrafish was raised.
- What was not a trend was how the solution with 0.25mg/mL of caffeine had a lower mortality rate than the 0.05mg/mL solution.
- This was not expected but could be explained by the low amount of variability in the data.

- Limitations of this experiment would be that the data set is relatively small, meaning that room for error in the data is very large.
- An error made when taking the data was that the experimental groups of fish were not placed in the experimental solution until the 48 hour mark as opposed to 24, as well as this, some of the fish were missing at 96 hours, eliminating any use of said data.

References

David, P., Berg, C., Tomasiewicz, H., Carvan,, M., Hesselbach, R., & Petering, L. (2018). ZEBRAFISH AS MODELS: STUDYING THE EFFECTS OF ENVIRONMENTAL AGENTS ON HUMAN HEALTH. Retrieved December 6, 2019, from University of Wisconsin - Milwaukee. Website: http://guides.library.uwm.edu/ld.php?content_id=2010971

Love, E., Bhattacharya, S., Smith, N., & Bhattacharya, S. (2010, June 24). *Effect of interpregnancy interval on outcomes of pregnancy after miscarriage: retrospective analysis of hospital episode statistics in Scotland.* Retrieved December 6, 2019, from Thebmj. Website: http:///www.bmj.com/content/341/bmj.c3967.short

Weng, X., Odouli, R., & Li, D. (2008). Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. Retrieved December 6, 2019 from American Journal of Obstetrics and Gynecology. Website: https://doi.org/10.1016/j.ajog.2007.10.803

YourGenome. (2014, November 17). *Why use the zebrafish in research?* Retrieved December 6, 2019, from YourGenome. Website: https://www.yourgenome.org/facts/why-use-the-zebrafish-in-research.

YourGenome. (2017, March 3). *What are model organisms?* Retrieved December 6, 2019, from YourGenome. Website: https://www.yourgenome.org/facts/what-are-model-organisms.