The Effect of Synthetic Versus Natural Caffeine on Zebrafish Embryo Development

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Introduction

The effects of caffeine intake during pregnancy are being debated and researched. The big question being posed by mothers-to-be across the nation is how much is too much? Caffeine is a stimulant that increases both heart rate and blood pressure, which is not recommended during pregnancy. The extent of the effects caffeine has on the fetus is still being researched ("Caffeine Intake During Pregnancy", 2017). The question posed in this study is whether synthetic or natural caffeine have more of an effect on the fetus. Zebrafish, Danio rerio, are ideal specimens to use in experimentation as model species due to their similarities to humans. They share 70 percent of their genes with humans, similar structures and have all the same organs as humans (Why use the zebrafish in research?). Zebrafish are also great test subjects because of their transparent embryos and the three day time period it takes to go from egg to larvae. (n.d.)

Caffeine was used on the experimental groups to see the effects. In humans, side effects of caffeine consumption include, insomnia, shakiness, headaches, diziness, anxiety, and more (Caffeine: MedlinePlus). Synthetic caffeine acts quicker, but causes a faster crash. Synthetic caffeine also lacks the vitamins and nutrients found in natural caffeine that gives it many of its health benefits (Caffeine: The Good, The Bad, The Alternatives). The source of natural caffeine used in this experiment was black tea. In addition to caffeine, tea contains many other chemical compounds that have health benefits synthetic caffeine does not (Chemical Compounds in Tea).

Hypothesis: If the zebrafish embryos are developed in natural and synthetic caffeine, then the development of the embryos exposed to synthetic caffeine will be more delayed than the natural because of the harmful effects and lack of nutrients in the synthetic caffeine.

Materials and Methods

- two well plates
- compound and dissecting microscopes
- dimethylene blue
- instant ocean solution
- synthetic caffeine
- one black tea bag
- hot plate, pipets
- two 250 mL beakers
- 1 L flask
- depression slides
- saran wrap
- an incubator
- 120 live zebrafish embryos

The following solutions were made: Instant ocean, 0.2mg/mL natural caffeine (black tea), 0.2mg/mL synthetic caffeine.

Four wells were labeled for each condition. At 25 hours post fertilization (hpf) 10 alive embryos were placed in each well with 3 mL of the corresponding solution. Some embryos were removed to make observations under the compound microscope, then placed back in the well and then placed in the incubator at 28.5°C. Days three through seven started by viewing the embryos under the dissecting microscope, removing dead embryos, and recording the stage of development. The well solutions were replaced. One embryo from each solution at each stage of development was removed and placed on a depression slide to be viewed under the compound microscope. The embryos were then returned to the wells and placed back in the incubator.

Observations and counts were not made for days five and six due to the weekend. The results were determined based on the number of embryos that lived and how their development was affected by the conditions. A T test was used to determine the significance of the results.

Results

Zebrafish embryos were tested over 169 hours to see if there was a difference in the development when exposed to natural versus synthetic caffeine. Both the synthetic caffeine and the natural caffeine had 0.2mg/ml concentration of caffeine. The independent variable was the type of caffeine for each group (blood natural, synthetic). The dependent variable was the amount of fish that hatched and died. The control was the embryos in the non caffeine habitat.

The results showed that the natural caffeine had a statistically significant effect on the hatching and death of the zebra fish embryos. The results showed that the synthetic caffeine did not have a statistically significant effect on the hatching and death of the zebra fish embryos.

Control 97hpf

Synthetic 97hpf

Data and Analysis

Using a T-test to compare number of hatched zebrafish in the control versus the synthetic shows there is a p-value of .3217, meaning the results are not statistically significant at a 90% confidence interval. Using a T-test to compare number of hatched zebrafish in the control versus the natural shows there is a p-value of .0009, meaning the results are statistically significant at a 90% confidence interval.

Using a T-test to compare number of alive zebrafish in the control versus the synthetic shows there is a p-value of .3601, meaning the results are not statistically significant at a 90% confidence interval. Using a T-test to compare number of alive zebrafish in the control versus the natural shows there is a p-value of .0068, meaning the results are statistically significant at a 90% confidence interval.

Discussion

The data collected in this lab does not support the hypothesis presented. The data shows that the natural caffeine had a much greater negative effect on the embryos than predicted. The cause of this is unknown and will require further testing. Using black tea as the source of natural caffeine presented errors in this lab. The black tea has other chemicals in it that could have caused the embryos death. Those chemical effects on developments are unknown. Using pure caffeine extracted from a natural source would give better results.

The results of the synthetic caffeine were not statistically significant and there was an error in the natural caffeine group that led to a compelling variable making this experiment inconclusive. This experiment failed to draw any conclusions regarding whether synthetic or natural caffeine has more of an effect on zebrafish embryo development. There is no evidence to state whether natural or synthetic caffeine is a better choice for pregnant women. More testing should be done to see the effects of pure natural and synthetic caffeine to ensure the best results.

Works Cited

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