The Effects of Caffeine on the Development of Zebrafish Embryos.
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Abstract
This experiment was done to test the effects of caffeine on developing zebrafish. Zebrafish are model organisms commonly used in scientific research because the embryological development of this species and humans are very similar. For five days, zebrafish were placed in wells of three different columns. Column one contained the Embryo Media Solution and was used as the control. Columns two through four contained different concentrations of caffeine. The zebrafish were observed over the five days and the number hatched and number dead were recorded. The results showed that high and low concentrations of caffeine proved to have a statistically significant effect on the hatch rate. There were many birth deformities present within all the concentrations. Knowing what caffeine can do to developing zebrafish embryos, humans can understand the effects of consuming caffeine while pregnant and its effect on developing human embryos.

Introduction
There is a major misconception when discussing caffeine: individuals feel caffeine has more beneficial effects than harmful effects. Health effects, caused by consumption of caffeine, can be even more harmful to a human embryo during pregnancy. In a recent study reported on by Medical News Today, researchers supported the statement that if pregnant women consume 300 mg of caffeine a day it may increase the risk of low birth weight babies and early death rate (Whiteman, 2015). The embryological development of zebrafish is very similar to that of humans, making these organisms an ideal species to test and compare a drug’s effect on the development of embryos. With the research gained one can then infer the effects of a drug on human embryo development.

It was hypothesized that if embryos were exposed to caffeine in the early developmental stages, then the drug would cause deformities and an increase in hatch rate among zebrafish.

Materials and Methods
● The materials used in this experiment included:
  - Caffeine solutions of 0.05 mg/mL
  - 1.15 ml pipette
  - Disposable pipette
  - Plate with wells
  - 28.5 Degrees Celsius
  - Dissecting and compound Microscope
  - Zebrafish and Zebrafish embryos
  - Gloves

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The embryos were separated from the adult zebrafish and cleaned before they were placed into the wells. 10 embryos were placed in each of the 12 wells. Not every well had 10 embryos at the end of the experiment because of death or accidentally sucking them up during the water change. The solutions were changed from the clean water of the embryos to any of the 3 chemical solutions. The first row consisted of the control which was the Instant Ocean/Embryo Media Solution, the second row was a concentration of 0.05 mg/mL, the third row was a concentration of 0.25 mg/mL, and the fourth row was a concentration of 1 mg/mL. The embryos were left to sit over night. The next day the rows were examined for dead embryos and all the water solutions were changed. The dead embryos, the living embryos, the hatched embryos, and the fry with birth deformities were recorded.

Results
In this experiment, the independent variable was the caffeine and the dependent variable was the hatch rate and mortality rate of the zebrafish embryos. The control was in column 1 of the well plate and was only filled with Embryo-Media Solution. The lowest concentration (0.05 mg/mL) was in column 2, the medium concentration (0.25 mg/mL) was in column 3, and the highest concentration (1 mg/mL) was in column 4. The hypothesis was that if developing zebrafish embryos were exposed to caffeine, then the zebrafish would have a slower hatch rate and would acquire birth deformities.

The results showed that the amount of caffeine had a statistically significant effect on the hatch rate of zebrafish, but not the mortality rate of zebrafish. All concentrations proved to not have a statistically significant effect on the mortality rate of zebrafish. The concentration (0.05 mg/mL) and the high concentration (1 mg/mL) were shown to slow hatch rate. However, the highest concentrations had the greatest affect on the mortality and hatch rate of zebrafish. There were developmental deformities, especially deformed spines, that were caused by the exposure to different concentrations of caffeine. The images displayed explains the birth defects among zebrafish placed in high concentrations of caffeine.

Discussion
The results supported the hypothesis that the zebrafish would acquire birth defects if exposed to caffeine, but did not support the hypothesis that caffeine would increase the hatch rate of zebrafish. Caffeine proved to only be statistically significant for the hatch rate in the low and high concentrations. When zebrafish were exposed to the low and high concentrations of caffeine the hatch rate greatly decreased, in comparison to the control. In the 1 mg/mL concentration of caffeine, only three zebrafish hatched out of a total of 28 embryos in the high concentration. In the 0.05 mg/mL concentration of caffeine, only eight zebrafish hatched out of a total of 29 total zebrafish in the low concentration. The 0.25 mg/mL was surprisingly healthier than the 0.05 mg/mL concentration. The 1 mg/mL concentration was the least healthy, which was expected.

There were deformities prevalent among the zebrafish in all concentrations. The zebrafish had bent spines and were underdeveloped. The deformity of bent spines was the most common deformity and was found in the low, medium and high concentrations of caffeine. Most of the zebrafish were underdeveloped.

Possible errors within the experiment could have been the misjudgement of whether the embryos were dead or alive. Also, there is a possibility that live embryos were removed before thorough examination. This experiment showed that caffeine can affect the embryological development of zebrafish, and is more than likely to affect the embryological development of humans. High dosages are much more dangerous to pregnant women than lower dosages. Possible future solutions to lessen the effects of caffeine, are to test medications that could prevent or decrease birth deformities in embryonic development.

References


Table 1: Final Number of Hatched Zebrafish. This table shows the number of hatched zebrafish throughout the entire experiment.

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References


Figure 1: Average Caffeine Exposure and Final Number of Hatched Fish
Figure 2: Zebrafish in a Higher Concentration of Caffeine. This zebrafish embryo has hatched prematurely and has a few deformities like smaller in size, curved spine, different sized eyes, and didn’t live long.
Figure 3: A Deformed Zebrafish in the Low Concentration of Caffeine. The figure shows that the spine of the zebrafish is curved and the fish is noticeably smaller and undeveloped, compared to the controls.