The Effects of Caffeine on Zebrafish Embryo Development

Sierra Hirsch

Biology

Ms. Laura Corado Koeppel

Mr. Ryan Schroeder

Waukesha South High School

March 10th, 2017

Abstract

The purpose of this experiment was to find the effects that caffeine has on zebrafish embryos. This information could be used to better understand the effects that caffeine has on human embryos. To test the effects of caffeine on developing zebrafish, embryos were placed in different concentrations of caffeine between fertilization and five days post fertilization. The embryos were observed every twenty four hours and the observations were recorded on paper. Notable results included the slow development of the embryos in all the wells and the curved spine and shorter tails in the well that had 1.0 mg/mL of caffeine. These results provided more information on the effects of caffeine on zebrafish embryos.

Introduction

This experiment was done to test the effects of caffeine on zebrafish embryos. Zebrafish, also known as *Danio rerio*, are freshwater fish that are model organisms due to their transparent embryos, rapid development and their development outside of the mother which allows for effective observation. Besides being a species that lives in the water, *Danio rerio* are actually very similar to humans in the way the embryos develop. However, a key difference is that zebrafish develop outside of the mother and take at least 48 hours to hatch while humans take approximately nine months to develop and be born.

The US Food and Drug Administration states that the average amount of caffeine consumed in the U.S is approximately 300 mg per person per day, which is around 2 to 4 cups of coffee. Caffeine is routinely consumed by many individuals around the world through teas, soft drinks, sodas and chocolate. There is a major misconception when it comes to caffeine: people believe that the benefits outweigh the risks. In reality, when consumed in large quantities, caffeine can cause many problems such as an upset stomach, insomnia, nervousness, restlessness, irritability, fast heartbeat and even muscle tremors. With so many people drinking copious amounts of coffee, human's need to know about the possible disadvantages and health concerns that they could possibly experience in the future.

Around 68-74% of pregnant woman consume caffeine at an average intake of 125-193 mg/day. When a pregnant woman drinks caffeine, the caffeine crosses through the placenta that surrounds the embryo. This can cause the embryo and mother to have strange sleep patterns, movements, rapid heart rate, higher chances of miscarriage, and feel the effects longer because it is still developing. Then when the child grows up, the embryonic caffeine exposure can cause adverse effects in adulthood. Caffeine is made for people to stay awake so if a woman consumes caffeine she could have trouble falling asleep and the embryo inside her would too.

Materials and Methods

Materials

- One plate with wells
- One petri dish
- One clear cup containing wide pipettes and small bore pipettes
- One bottle of Embryo Media/Instant Ocean
- One bottle of 0.05 mg/mL caffeine solution

- One bottle of 0.25 mg/mL caffeine solution
- One bottle of 1.0 mg/mL caffeine solution
- One waste beaker for dead embryos and waste solution
- One stereoscope
- One light microscope
- One incubator set at 26 through 28 degrees Celsius
- Depression Slides
- One permanent marker
- Zebrafish embryos

Methods

In the well plate all the wells were filled up with one mL of the embryo media using a wide pipette. In the second well one mL of 0.05 mg/mL caffeine solution was added, the third well one mL of 0.25 mg/mL caffeine solution, and the fourth well 1.0 mg/mL solution. Ten embryos were placed in each of the wells using a small bore pipette. The exact number of embryos was recorded. Observations were recorded on the students' data sheets. The embryos were then placed onto a tray that was placed in the incubator that was set at 26 through 28 degrees Celsius.

On days two and three the well plate was removed from the incubator for observation. The embryos that were dead were placed into the waste beaker using a small bore pipette. The old solutions were drained with a small bore pipette and replaced with the appropriate new solutions of caffeine using a wide pipette. The embryos were then counted and recorded along with fish that were hatched too. The embryos were then placed individually on a depression slide that was viewed underneath a light microscope. Observations were recorded in students' papers. All embryos were placed back into their appropriate wells and then placed inside the incubator.

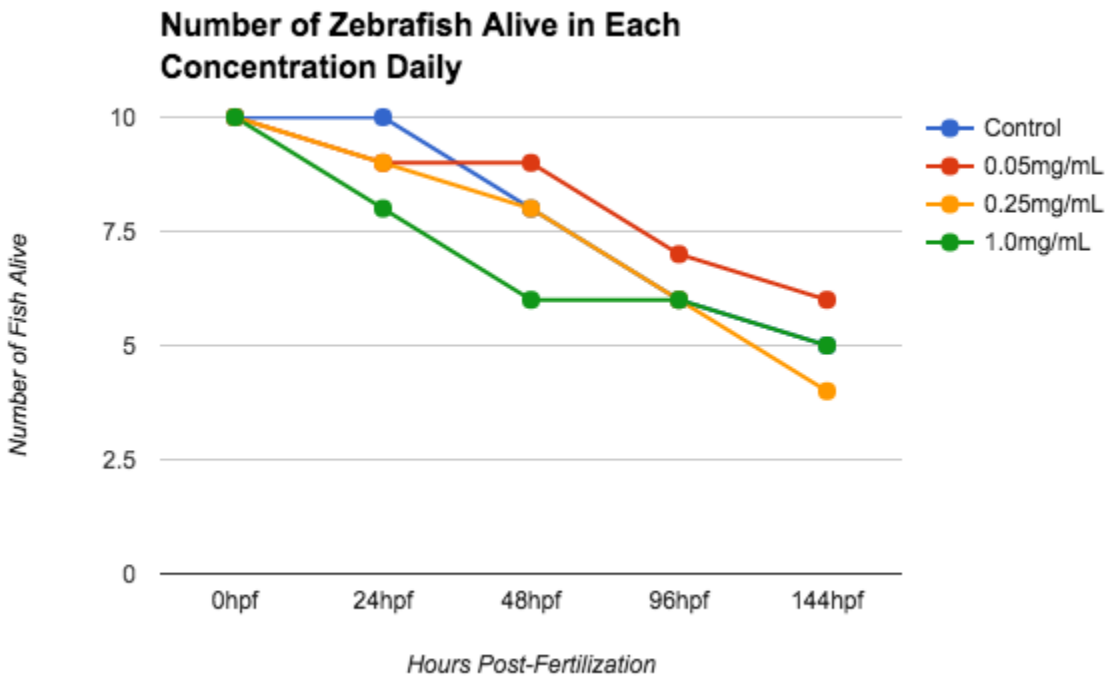
On day four the well plate was removed from the incubator for observation. The embryos that were dead were removed and placed into the waste beaker using a small bore pipette. Old solutions were drained and the appropriate new solutions were added using the wide pipette. The embryos and fish were then counted and recorded. The embryos were then placed individually onto depression slides that were viewed underneath a light microscope. The observations were recorded in students' papers. All embryos were placed back into their appropriate wells and then placed inside the incubator. On day five the well plate was removed from the incubator for observation. The embryos that were dead were removed and placed into the waste beaker using a small bore pipette. Old solutions were drained and the appropriate new solutions were added using the wide pipette. The embryos and fish were then counted and recorded. The embryos were then placed individually onto depression slides that were viewed underneath a light microscope. The observations were recorded in students' papers.all embryos and fish were then moved from their wells and placed into the waste beaker. The embryos and fish were then euthanized by freezing at the end of day four.

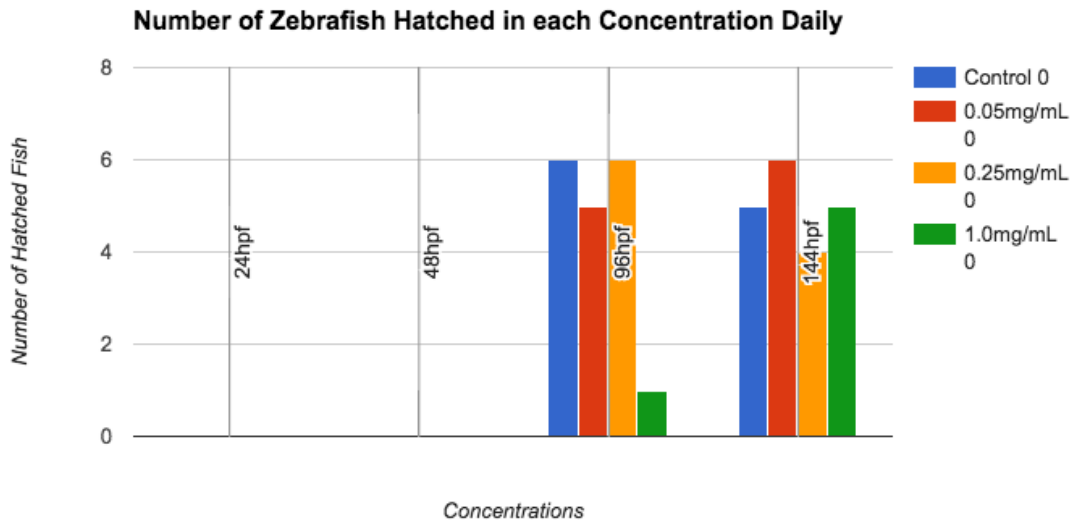
Results

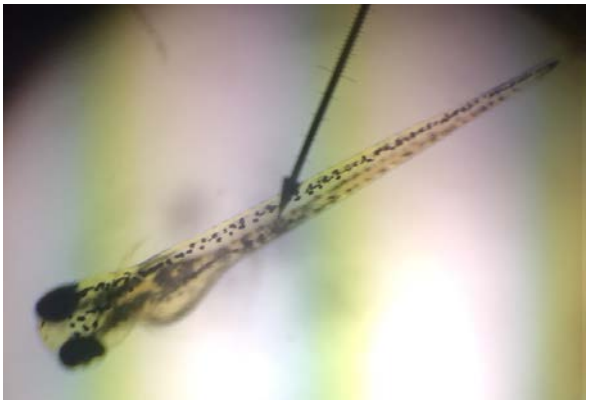
This experiment was conducted in order to discern the effect of caffeine on zebrafish embryos. It was hypothesized that the embryos exposed to high concentrations of caffeine would have faster heartbeats, be smaller in size, and would die more rapidly. The embryos were divided by wells and exposed to different concentrations of caffeine. The independent variable was the concentration of caffeine and the dependent was the number alive, number hatched, and the appearance and behavior of the embryos.

The embryos that were placed in caffeine did not live as long. More of the embryos died in the caffeine wells. Both the control and caffeine affected embryos did not hatch until 96 hours post fertilization and the embryos in each well developed at a slow rate and none started hatching until 96 hpf. Spots were not visible until 48 hpf in both controls and caffeine-exposed embryos. Some of the embryos that were in the caffeine concentrated wells had deformations such as spine abnormalities. However, in the 1.0 mg/mL well the embryos had shorter tails and a spine deformity where the spine was somewhat curved. Another difference between the control and caffeine embryos was the yolk sacs were larger in the caffeine embryos starting at 48 hpf. Some embryos in the 1.0 mg/mL concentration did not hatch by the time the experiment was terminated.

The results partially supported the initial hypothesis, as well as providing further detail about caffeine's impacts on development. Most of the desired information that was recorded was found in the results shown.







Control - 96 hpf (Day Four)

1.0 mg/mL - 96 hpf (Day Four)

0.25 mg/mL - 96 hpf (Day Four)

Discussion

Some notable results from the experiment were the slow development of the embryos in all of the wells, the shorter tails and spine deformities in the well 1.0 mg/mL, and the larger yolk sacs in comparison to the controls. This provided more information on how the drug caffeine affects the development of zebrafish embryos.

It is possible that the caffeine exposed-embryos could have developed more deformities through the moving and handling of the experimenters. This is unlikely though because the deformities in the caffeine were a little more severe and complicated then what handling and moving the embryos could cause. Also the control embryos were handled daily as well and there were no severe problems with them. Using pipettes to the move the embryos to place them on the depression slides would likely not cause an embryo's tail to be shorter or cause slower development. The incubator on the first day was a couple of degrees below 26 degrees celsius so some results may have been affected by that drop in temperature.

This experimenter did not locate any research that discussed spinal deformities resulting from caffeine exposure for human embryos. There are some things however, they cannot see and record when looking at the zebrafish embryos, like social disorders, mental disorders, and the embryo's sleep patterns because that is a side effect of caffeine. This is important because embryos who are exposed to caffeine at a young age could have long term mental problems when they grow up that they did not know could happen because of the mother's consumption of caffeine during pregnancy.

References

American Pregnancy Association. *Caffeine Intake During Pregnancy.* Sept. 1st, 2016.

Mayo Clinic. *Caffeine: How Much Is Too Much?* n.d.

University of Oregon. *Zebrafish FAQs*. January 2nd, 2013.