THE EFFECTS OF CAFFEINE ON ZEBRAFISH EMBRYO DEVELOPMENT

VAISHNAVI

PRE-AP BIOLOGY

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Abstract

This experiment was conducted in order to reinforce and verify past research on the effects of caffeine on zebrafish. To test the effects of caffeine on developing embryos, Zebrafish embryos were placed in varying concentrations of caffeine between fertilization and five days post-fertilization. The embryos were examined every twenty four hours. Notable results include the irregular heart rate, small size, the physical abnormalities, general inactivity, and the slow development of the caffeine-affected embryos. All of these should be similar to defects found in caffeine-affected human embryos. These results provide a lot more research to support the arguments against caffeine.

Introduction

This experiment was done to test the effects of caffeine on zebrafish embryos. Zebrafish(*Danio rerio)*, are freshwater fish that are model organisms due to their transparent embryos, rapid development and their development outside of the mother. This allows scientists to see the model development of the embryos without any hindrances. Also, Zebrafish are more closely related to humans than commonly used invertebrate models such as fruit flies(*Drosophila*) and worms(*Caenorhabditis elegans*) due to their status as vertebrates. Aside from 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 incredibly quickly (24 hours post fertilization) while humans take approximately nine months to develop and be born. Due to these reasons, Zebrafish are the most commonly used model for all vertebrates, including humans.

Caffeine is a central nervous system stimulant and is also the world's most widely consumed psychoactive drug. It is a neurotoxin alkaloid. Caffeine is not regulated in most of the world, and therefore can be obtained very easily. 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, coffee, and chocolate. There is a major misconception when it comes to caffeine; many 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, irritability, insomnia, nervousness, restlessness, irritability, a fast heartbeat, and even muscle tremors. With so many people drinking copious amounts of coffee, we need to know about the possible disadvantages and health concerns that we could possibly run into in the near foreseeable 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 passes through the placenta that surrounds the baby. This can cause the baby and the mom to have strange sleep patterns, movements, rapid heart rate, higher chances of miscarriage and could seriously impact the embryo as it will feel the effects longer. When the child grows up, the embryonic caffeine exposure could also cause many adverse health problems in adulthood which would not let the person perform to the best of their ability in life. Caffeine is made for people to stay awake so if a mother consumes caffeine she would have trouble falling asleep and her baby would too.

The purpose of this experiment is to reinforce and verify the results of other research on the effects of caffeine on Zebrafish embryos. This can be linked to studies of the consequences that caffeine has on human embryos.Through the information we found, we expect to see a correlation between the two. It was hypothesized that the embryos exposed to high concentrations of caffeine would have faster heartbeats, be smaller, hatch later, and have a

higher mortality rate than the control fish. The experiment found that caffeine-affected zebrafish developed more slowly than the control, but with abnormalities in the backbone, heartbeat, size, hatching rate and development.

Materials and Methods

Materials

- One bottle of Instant Ocean/ Embryo Media
- 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 beaker for dead embryos and waste solution
- One permanent marker
- One minimum bore 1.5mm pipette for transferring the embryos to and from the container
- One 1 mL wide bore pipette
- One well plate
- One incubator set at 28 degrees Celsius
- Depression slides with cover slips
- One stereoscope
- One light microscope
- 40 zebrafish embryos

Methods

The four wells on the plate were filled with 1 mL of Instant Ocean/ Embryo Media solution using the wide bore pipette. The second well was filled with 1 mL of 0.05 mg/mL caffeine solution, the third with 1 mL of 0.25 mg/mL caffeine solution, and the last with 1 mL of 1.0 mg/mL caffeine

solution. 10 embryos were placed in each well using the minimum bore pipette. The exact numbers of live embryos were recorded on the student's data sheets using the stereoscope. Dead embryos were discarded into the waste beaker. Embryos were observed and counted in the plate using stereoscopes and individually on depression slides under the light microscopes. Observations were recorded on the student's data sheets. The embryos were returned to their respective wells in the plate and the plate was placed in the incubator at 28 degrees Celsius. On days two and three, the plates were removed from the incubator for observation. The dead embryos were removed from the wells and placed into the waste beaker. The solutions were drained and replaced with new solutions of the appropriate concentrations of the caffeine. The old solution was discarded into the waste beaker. The living embryos were counted along with the hatched fish. The embryos in each well were observed under the stereoscope, with all the observations recorded onto the data sheet. Individual embryos were then placed on the depression slides and observed under the light microscope. All the embryos were returned to their well on the plate, and the plate was placed into the appropriate 28 degrees Celsius incubator. On day four, the plate was removed from the incubator and the solutions drained. The living embryos and hatched fish were counted. All observations were made and recorded on the data sheets. The embryos and the fish were removed from the well plate and placed into the waste beaker. The embryos were euthanized by freezing at the end of day 5.

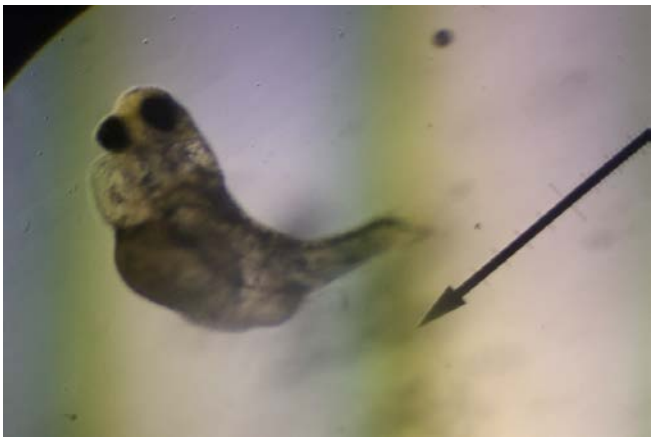
Results

The experiment was conducted in order to verify previous research involving embryonic caffeine consumption, caffeine dependency and the future impacts of caffeine on an embryo's development. It was hypothesized that the embryos exposed to high concentrations of caffeine would have faster heartbeats, be smaller, hatch later, and have a higher mortality rate than the

control fish. The embryos were separated into different wells on a well plate, with the control in only embryo media/ Instant Ocean solution, and the others in successively denser concentrations of caffeine, the independent variable being the concentration of the drug, and the dependent the number and the physical habitat of the living fish.

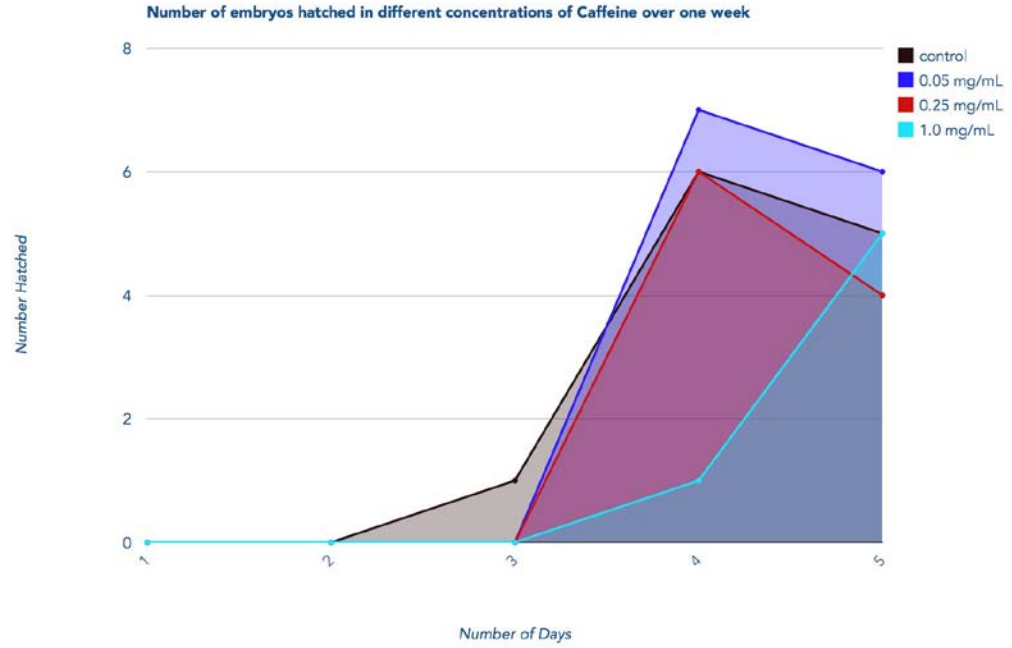
The caffeine-affected embryos generally hatched later than the control, were smaller as a whole, and had visible deformations in the backbone, yolk sac, tail, and heart. One clear deformation is the bent spine and short tail of the embryo in the 1.0 mg/mL caffeine solution compared to the control after 144 hours post fertilization. Another difference between the control and drugged embryos in 1.0 mg/mL caffeine solution on 144 hours post fertilization, is that the yolk sac is visibly larger on the 1.0 mg/mL caffeine-affected embryos. Most development slowed down to a stop on the caffeine-affected fish by 48 hours post fertilization, and they also became increasingly lethargic and inactive at this point. The heartbeat of the caffeine-affected fish was also more erratic and slower that the controls', and was not at regular intervals. The drugged fish were half the size of the control and rarely moved after hatching.

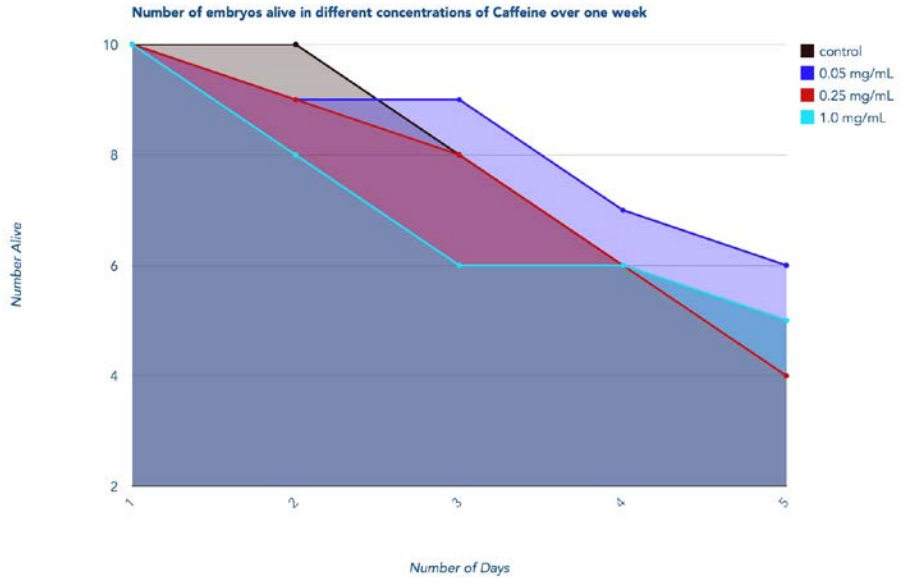
The results supported the initial hypothesis, as well as provided further information about caffeine's impact on embryonic development. Most of the desired data was found in the results, although zebrafish cannot provide information on cognitive skills, motor skills, societal issues and mental disabilities which caffeine can be a cause of.



1.0 concentration, Hour 144

Control, Hour 144


Number of embryos hatched in different concentrations of Caffeine over one week
control
0.05 mg/mL
0.25 mg/mL
1.0 mg/mL
Number Hatched
Number of Days


Number of embryos alive in different concentrations of Caffeine over one week
control
0.05 mg/mL
0.25 mg/mL
1.0 mg/mL
Number Alive
Number of Days

Discussion

Some notable results include the slow development, general inactivity, physical deformations, and smaller size of the caffeine-affected fish. The deformations could be linked to the slow development and hatching of the caffeine-affected fish. The drugged fish also had shorter tails than the control fish. The zebrafish affected by caffeine had many more deformities than the control fish and were smaller as a whole. The higher the concentration of caffeine, the more deformities were present in the fish. This fact provides more solid evidence that caffeine is the cause of the deformities and slow development. The deformities and slow development could also have occurred due to the incubator being set a few degrees lower than it was supposed to be, but the lower heat should have affected the control too and not only the drugged embryos, so this concern is not valid.

The results support previous research conducted on embryonic caffeine exposure and zebrafish. The experiment showed that caffeine leads to premature birth, physical deformities, movement issues and heart problems, most of which have been linked to caffeine. The results also provide further evidence that the concentration of caffeine can affect the outcome, as problems seen became more severe in higher doses of the drug.

The erratic heartbeat of the caffeine-affected zebrafish could also be related to the faster heartbeats in human babies, which is a consequence of caffeine. The fish in the 1.0 mg/mL caffeine solution stopped moving at around 144 hours post fertilization. The fact that embryos were smaller and more inactive in higher concentrations of caffeine gives more proof that caffeine is the cause of slow development.

References

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http://www.neuro.uoregon.edu/k12/FAQs.html>

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