The Effect of Caffeine on the Mortality Rate of *Danio rerio* Embryos

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Abstract

In a study of nearly 51,000 pregnant women, 44% of them consumed 50-199 mg of caffeine daily, despite controversy over whether or not it is safe to drink caffeinated beverages while pregnant (Papadopoulou, Botton, Brantsæter, et al., 2018). To determine the effect of caffeine on embryonic development, an experiment was conducted. Due to ethical issues with performing this experiment with human embryos, the model organism *Danio rerio* (zebrafish) was tested on instead. Thus, this investigation identified the effect that caffeine has on the mortality rate of zebrafish embryos, which can then be used to predict the effect that the substance would have on human embryos. In the investigation, approximately ten zebrafish eggs were placed in each of twelve wells in a well plate. Then, the wells were filled with either control solution, solution containing 0.05 mg/ml caffeine, or solution containing 0.25 mg/ml caffeine, with each solution being present in four wells. At 24 hour intervals, the number of surviving zebrafish in each well were counted. At 72 hours post-fertilization, 92.31% of embryos in control solution were alive, 76.47% of embryos in the 0.05 mg/ml caffeine solution were alive, and 80.65% of embryos in 0.25 mg/ml solution survived. The results demonstrate that the presence of caffeine increases the mortality rate of zebrafish embryos, and thus is harmful to their survival. Since zebrafish can be used to model how substances can affect humans, the conclusion can be drawn that it is unsafe for women to consume large quantities of caffeine while pregnant, as the substance has potential to be harmful to the developing embryo.

Background Information

Zebrafish as Models

Zebrafish will be used for this test because they are excellent model organisms (Khan, Alhewairini, 2018). In fact, zebrafish are often used for the study of cancer, genetics, and

various human illnesses because of how good of a model they are for these conditions (Khan, Alhewairini, 2018). Model organisms are species that bear many similarities to humans in genes and are used in laboratory experiments to learn more about humans (yourgenome, 2017). These model organisms are used because it is not ethical to do certain tests on human subjects, so it is best to run tests on subjects that are similar to humans (yourgenome, 2017). For example, biologists can cause mutations in organisms such as zebrafish and observe their effect, or change factors of an organism's environment in order to study how pollutants may impact the life of the organism (yourgenome, 2017). Zebrafish are an excellent model organism for studying development because they develop quickly, with an embryo developing as much in a day as a human embryo will in a month, and the zebrafish embryos are nearly transparent, and thus it is easy to observe development inside the embryo (yourgenome, 2014). Zebrafish are specifically excellent models for the development of human embryos because 70% of human genes can be found in zebrafish (Burke, 2016). Additionally, zebrafish have many of the same organs and other body parts as humans, which makes it possible to model many conditions and diseases found in humans in a zebrafish (Burke, 2016). Thus, using the zebrafish model, it can be determined how caffeine would affect the development of a human embryo.

Science of Caffeine

According to the Mayo Clinic (2017), caffeine is frequently used by people for the purpose of avoiding tiredness and improve focus and alertness. When the substance is consumed, it tricks the parts of the body that induce sleep into keeping the body awake, hence why it is frequently used to help people stay awake and alert (National Sleep Foundation, 2019). Caffeine has this effect on the body by blocking receptors in the brain that cause drowsiness (Brain, Bryant, Cunningham, n.d.). Human bodies naturally produce a chemical

called adenosine, which can bind to adenosine receptors in places of the body such as the brain (Brain, Bryant, Cunningham, n.d.). Adenosine bonding to these receptors slows down nerve activity in the brain, which causes the body to feel tired or drowsy (Brain, Bryant, Cunningham, n.d.).

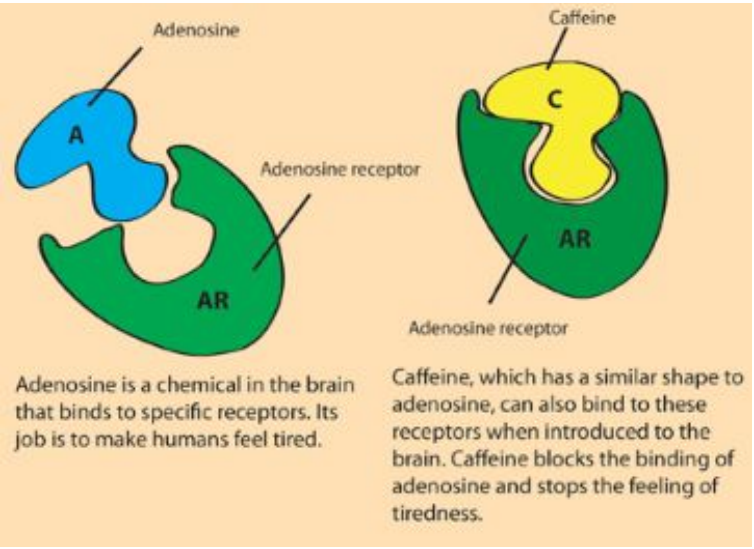

Figure 1:

Graphic showing caffeine's ability to block adenosine receptors(SciTech, 2013).

However, as shown in Figure 1 above, caffeine bears a similar shape to that of adenosine, and so when it is present in the body, caffeine can bind to the adenosine receptors instead of the adenosine (SciTech, 2013). Because the adenosine is not inhibiting the receptors, the brain does not receive signals to slow down nerve activity, which prevents the feeling of tiredness (SciTech, 2013). When consumed in large doses, caffeine also induces apoptosis in cells within the body and can lead to the development of conditions such as osteoporosis (Lu, Lai, Chan, 2008, p.13). Due to the effects that caffeine has on the function of the brain and the potential damage that it can cause to the body, it is predicted that caffeine will also have

damaging effects on zebrafish embryos, and exposure to caffeine will likely result in the deaths of more of the embryos that are exposed to caffeine.

Investigation

When the experiment was conducted, caffeine was added to the water in which zebrafish developed. By adding caffeine to the solution that the zebrafish develop in, it can be determined the likely effect that caffeine would have on the development of human embryos. Thus, the purpose of caffeine in the experiment was to determine what effects caffeine has on the mortality rate of zebrafish embryos during development. The data collected on the mortality rate of zebrafish in various concentrations of caffeine provides a good idea about how the presence of caffeine can affect the development of human embryos.

Materials and Methods

Participants

Starting on Tuesday, November 5, 2019, the Honors Biology class at Greendale High School participated in an investigation of the effect of caffeine on the growth and development of zebrafish embryos. Between November 5 and November 8, students exposed zebrafish to varying concentrations of caffeine and collected data on how many fish were alive and how many were hatched each day. Data was collected between 1:51 P.M. and 2:35 P.M. each day of the investigation.

Materials

During this investigation, materials used include the following: beaker for disposal of waste, flask containing Instant Ocean solution, bottle containing caffeine for making diluted

solutions, plate with wells for the eggs and solution, 1 ml disposable pipettes, fine-tip pipettes, a dissecting microscope, bottles containing either 0.05 mg/ml or 0.25 mg/ml caffeine solution, funnels for creating the solutions of varying concentrations, and tape and a pencil for labelling the bottles.

Experiment Design

During this investigation, the concentration of caffeine in solution that the embryos were exposed to was changed in order to measure the effect that caffeine had on how many zebrafish embryos lived and hatched. Four wells containing ten embryos each were filled with control solution that contained no caffeine. Four wells were filled with 0.05 mg/ml caffeine solution and the remaining four were filled with 0.25 mg/ml caffeine solution, with ten embryos in each well. In total, we used 120 embryos, 40 of which as a control group and 80 of which that we experimented with.

Procedure

First, 2.11 ml of caffeine was combined with 40 ml Instant Ocean to create a bottle of 0.05 mg/ml caffeine solution, and then 13.3 ml of caffeine was mixed with 40 ml Instant Ocean to create a bottle of 0.25 mg/ml caffeine solution. Then, the wells of the plate were labelled for identification of each well, and then each well was filled with ten embryos and approximately 1 ml of Instant Ocean solution. The plate was then covered and placed into an incubator. The following day, the plate was removed from the incubator and the number of alive embryos as well as the number of hatched zebrafish were counted. Then, the Instant Ocean was removed from each well. Four of the wells were refilled with Instant Ocean, four wells were filled with 0.05 mg/ml caffeine solution, and the remaining four wells were filled with 0.25 mg/ml caffeine

solution. The embryos were then observed under a dissecting microscope and pictures were taken of the microscope images. The plate was then placed back into the incubator. The following day, Thursday at this point, the plate was removed from the incubator, and the process of the previous day was followed; the number of alive embryos and hatched fish were counted, the old solution in the wells was replaced by the same type of solution(control, 0.05 mg/ml, or 0.25 mg/ml caffeine), and the fish were once again observed under the dissecting microscope. The plate was once again placed in the incubator. The next day, the final day of the lab, the plate was removed from the incubator and the same process for collecting data and waste removal was followed from previous days, except new caffeine solution was not put into the wells. Embryos were observed under a microscope, and then all waste and leftover caffeine solution was disposed of(University of Wisconsin-Milwaukee, 2018, p.41-42).

Results

Summary of Data

During this investigation, four of the twelve wells in the plate served as the control group, and were filled with control solution that contained no caffeine. The experimental group consisted of four different wells that were filled with a solution containing 0.05 mg/ml caffeine in addition to the remaining four wells, which were filled with 0.25 mg/ml caffeine solution, making a total of eight wells in the experimental group. In total, 26 fish were exposed to control solution, 34 fish were exposed to 0.05 mg/ml caffeine solution and 31 fish were exposed to 0.25 mg/ml caffeine. In all of the wells containing control solution combined, 92.31% of the fish survived, compared to 76.47% survival rate in the 0.05 mg/ml caffeine solution and a 80.65% survival rate in the 0.25 mg/ml solution.

Tables and Figures

The Effect of Exposure to Varying Concentrations of Caffeine on the Development of Zebrafish Embryos

Hours Post Fertilization	Average Survival Rate of Embryos(%)		
	Control Group	0.05 mg/ml Caffeine	0.25 mg/ml Caffeine
24	100	100	100
48	100	100	100
72	92.31	76.47	80.65

Figure 2:

Table showing that as the number of hours post fertilization increased, the average survival rate of embryos decreased. Additionally, as a greater concentration of caffeine was in the solutions, less fish survived.

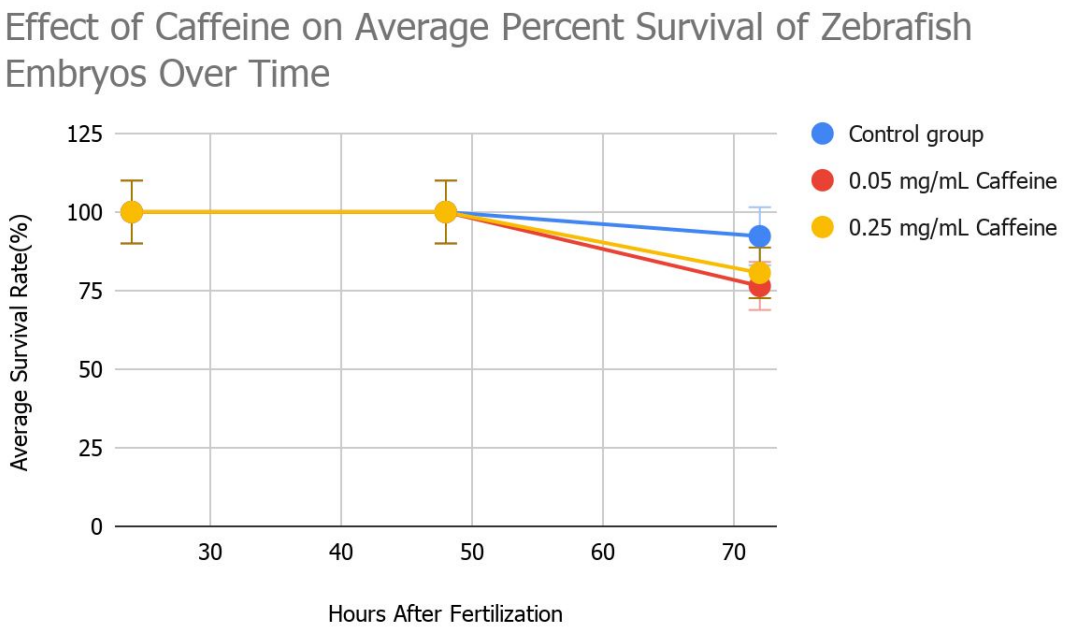

Figure 3:

Graph showing that as time increases, the average survival rate of the fish decreases.

Additionally, at greater concentrations of caffeine, more fish died.

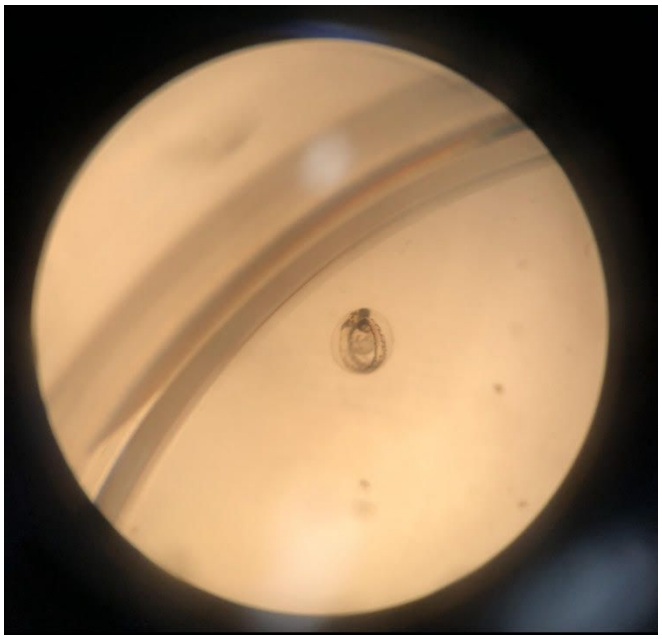

Figure 4:

Image showing a living zebrafish embryo in the control solution 48 hours after fertilization.

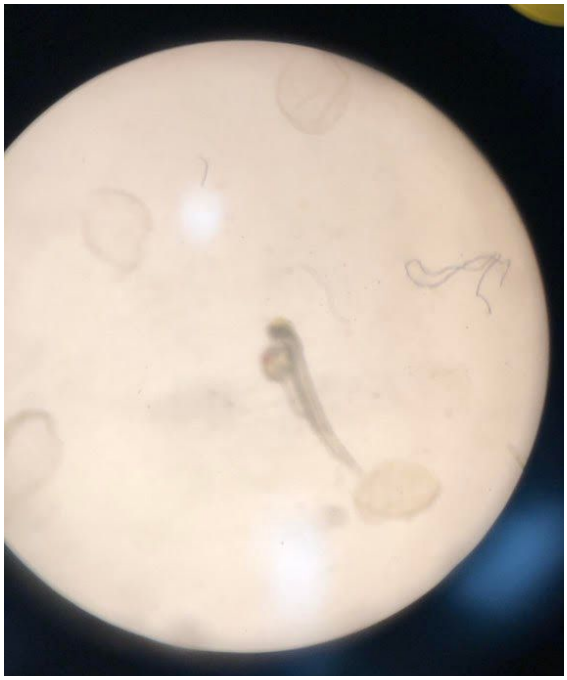

Figure 5:

Photograph of a living, hatched zebrafish embryo surrounded by dead embryos in 0.05 mg/mL caffeine solution 72 hours after fertilization.

Statistical Analysis

The investigation showed that there was a higher mortality rate in the experimental group of fish that were exposed to caffeine than those in the control group that were not exposed to the chemical over the course of a 72 hours. In order to confirm the statistical significance of the data that was collected, a t-test was performed with the data. The first t-test was performed comparing the control group to the group that was exposed to 0.05 mg/ml caffeine solution. This test returned a p-value of 0.56. The second t-test compared the control group to the 0.25 mg/ml caffeine solution. This test returned a p-value of 0.61. These t-tests were conducted using the website GraphPad(GraphPad, 2019). Despite the data showing a greater mortality rate of zebrafish in the experimental groups, the t-test showed that the data is

not statistically significant. This suggests that although the data presented a clear indication that caffeine does have an effect on the development of zebrafish embryos, a larger sample size would need to be tested in order to achieve fully reliable information and confirm this relationship.

Discussion

Significance of Investigation

Understanding how caffeine affects the development of zebrafish embryos helps to better understand how caffeine may have a similar effect on human embryonic development. Zebrafish share 70 percent of their DNA with humans (yourgenome.org, 2014), and so by testing for the effects of caffeine on zebrafish embryos, the effects of the substance on human embryos can be accurately predicted. Thus, the significance of caffeine on the development of zebrafish will allow for better understanding of how frequent exposure to significant quantities of caffeine can interfere with the development of human embryos.

Conclusions

The data provided by this investigation suggests that the presence of caffeine does have a negative effect on the development of zebrafish embryos. After 72 hours, there was a greater percentage of zebrafish alive in the wells containing control solution than in the other wells containing caffeine, with a 92.31% survival rate in control solution, as opposed to a 76.47% survival rate in 0.05 mg/ml caffeine solution and a 80.65% survival rate in 0.25 mg/ml caffeine solution. There is a clear decrease in survival rate from the control group to both experimental groups. Thus, this data supports the hypothesis that caffeine exposure results in a higher mortality rate of zebrafish embryos. However, although the graph represents data over the

course of three days, the fish were only exposed to caffeine from 48-72 hours post fertilization, and thus there is little data to go off of. Initially, it was intended that the fish would also be observed at 96 hours post fertilization, however the wells containing 0.25 mg/ml caffeine contained neither solution nor fish at this point, and many of the remaining full wells were infested with parasites. For this reason, data could not be collected at 96 hours post fertilization, which resulted in less data to draw conclusions from. Despite this, the 24 hours for which data could be collected was enough to demonstrate the negative effect of caffeine on embryonic development.

An experiment conducted by Close (2019) investigated the effects of caffeine on developmental problems in zebrafish. Her lab found that zebrafish exposed to caffeine as embryos developed abnormalities, including bent tails, that made it difficult for the fish to swim. Deformed spines were also observed (Close, 2019, p.6-7). Additionally, her experiment found that less zebrafish survived in higher concentrations of caffeine than in low concentrations or in control solution (Close, 2019, p.6-7). The data collected from the experiment conducted by Close aligns with the data presented in this paper, with a greater mortality rate of the zebrafish in higher concentrations of caffeine. Thus, it can be concluded from several experiments that caffeine is dangerous for developing zebrafish embryos. Due to the genetic similarities between zebrafish and humans, we can make conclusions about how a human system may be affected by caffeine based on the effect the substance has on zebrafish embryos. This means that it can be concluded that it is dangerous for human embryos to be exposed to caffeine, and so women should try to avoid consuming caffeine while pregnant to ensure the safety of their child.

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