Abstract

This experiment was performed to examine how the effects of caffeine would affect the growth rate of zebrafish embryos, and developing fetuses. By looking at the zebrafish and fetus growth rates, it's possible that there could've been something in common between them. Some of the methods used while performing this experiment included checking the growth rate of the embryos daily, and take pictures. This was important so data of their growth could be recorded, since it was the main idea. After all of the data was collected, twelve embryos in 0-0.05ml of caffeine hatched and three died, out of 20. In 0.25-1 mg/ml of caffeine, only three embryos out of twenty hatched, and nineteen died. So there was a significant hatch and death rate difference between the different amounts of caffeine. But overall, the death rate related to the fetuses more than growth rate. Information from sources stated that if a pregnant woman intakes too much caffeine, they're more likely to have a miscarriage, where the baby dies before its born. And from the zebrafish experiment, it was learned that a high amount of caffeine will cause an increased death rate. So in relation, caffeine can cause an increased chance for a miscarriage, and an increased death rate in zebrafish.

Introduction

Caffeine is a compound that is mainly found in coffee plants and tea, and is usually used as a stimulant to keep one awake. It's also known to be addicting, and it's the most common drug throughout the whole world. This experiment that was performed came from the base idea of how developing zebrafish embryos would be affected by caffeine over the course of 72 hours. The purpose of this experiment was to determine how caffeine affecting the development of zebrafish would relate to how caffeine would affect the development of fetuses. A few key ideas from sources included that too much caffeine can cause a miscarriage, it can raise blood pressure
and heart rate, and it also stimulates the brain to keep you awake. Some information on how it affected zebrafish included that it would slow the growth rate of embryos, yet increases the death rate of the embryos the more caffeine there would be. The concluded hypothesis was, “If caffeine stunts human growth, then it may also do the same to zebrafish growth and development.”

Materials and methods

Materials: Tray with wells, Sharpie, Disposable pipette, Stock solutions of Caffeine (0mg/ml, 0.05mg/ml, 0.25mg/ml, and 1mg/ml of Caffeine), Beaker, Embryo Media Solution, 28.5 degrees Celsius incubator, Dissecting microscope, and a few Disposable pipettes at least 1.5 mm for transferring eggs to observation well.

For a safety precaution during the lab, make sure to wear gloves and if needed, safety glasses to avoid contact with embryos and solution.

Note: After day one, caffeine would be switched out and replaced everyday to remain fresh.

On day one, the materials listed above were setup to use. Four wells on the tray were set up and used for different observation and testing areas for the zebra fish embryos, each well containing a different amount of caffeine (listed above). Each well started with ten alive embryos, which from observations; had not hatched yet and were subtle with movement. The embryos were placed in the wells using the 1.5 mm pipette, and caffeine solutions were placed in every well also using a pipette. Pictures were also taken with a camera, to record results and data visually.

On days two and three, the same procedure would be completed. First, observe the fish with the compound microscope. Around this time a few fish would usually hatch and swim around every so often, usually in sudden bursts. It's possible for some that a few fish might have died, but the majority should've remained alive. After the fish would be observed, the caffeine would be checked and replaced if not already done. Any data would be recorded, and pictures would be taken if needed. After, the work
space would be cleaned up, and the trays containing the fish would be put back in their incubator.

On day four, the same steps would be finished, but a few more added. Like days two and three, observe the fish with the compound microscope, and record data. Caffeine would be replaced if hadn't already. Pictures would be taken for the last portion of data, and work space cleaned up. Most fish would be hatched by now, but also quite a few dead, depending on what compound used for the experiment. Also, fish movements would again include sudden bursts of motion, but sometimes also swimming regularly. But the fish usually remained still unless the well was moved. Using the pipette and a beaker, any dead fish would be extracted and thrown away. All remaining alive embryos and fish would be extracted using a 1.5mm pipette and moved to a fish tank. Work space would then be cleaned up to finish the day.

After the experiment was finished, students would then work on project, which included the Chi Square Analysis. Data would be filled out, and students would then look for their “degree of freedom”. Students would then find their degree of freedom and accept or reject the null hypothesis based on their results. If the null hypothesis was rejected, the experiment was done correctly. If it was accepted, the experiment could’ve had some issues.

Results

The research inquiry question was, “How will caffeine affect zebrafish embryos over the course of 72 hours?” The goal was to try and learn if caffeine would affect the development of zebrafish. So the hypothesis was that if caffeine stunts human growth, then it may also do the same to zebrafish growth and development. In the experiment, caffeine was replaced every day, and with the dissecting microscope, the embryos were observed daily to see how much they had developed, and data would be recorded.

The independent variable was the amount of caffeine used, since it would be affecting the dependent variable. The dependent variable were the zebrafish, since their growth rate and development was being changed and recorded from being affected by the amount of caffeine. The control
variable were the wells, since they enclosed the zebrafish in a small space, and were needed for the experiment to take place. Secluding the fish in a small space could affect their growth rate, since there’s not a lot of area for them to move around in, and there’s less oxygen in the smaller space. So being in a small space with a little amount of oxygen could affect their growth rate, because the fish may not be getting enough oxygen.

The experiment lasted for 72 hours, and every 24 hours data would be recorded, so there would be in total four days to record data, including data from the starting day. There were also four different solutions of caffeine used, (0mg/ml, 0.05mg/ml, 0.25mg/ml, 1 mg/ml) and four wells to put the separate solutions into. Forty embryos were tested, ten in each well. At the beginning of the experiment, most of the embryo were healthy, but none of them would be hatched, all still in their eggs. On day three, four embryos hatched in well one, with 0mg/ml of caffeine. Three embryos hatched in well two, with 0.05mg/ml of caffeine. Two embryos hatched in well three, with 0.25mg/ml of caffeine. And one embryo hatched in well four, with 1 mg/ml of caffeine. No embryos died in any of the wells. The hatched zebrafish were long and skinny, and there was a sac was right below the eyes for all of the fish. The fish were also able to swim, usually sudden bursts of quick motion. On day four, seven embryo total hatched in well one, and eight remained alive. In well two, five embryo total hatched while nine remained alive. In well three, two embryo hatched total, but one remained alive. One of the hatched embryos hatched from the previous day but died. In well four, one embryo hatched, but all of the embryo died. The fish in all of the wells were different from one another, more specifically in how deformed and changed they were. The fish in wells one and two were both pretty healthy and mobile, since there was a very low amount of caffeine in the two wells. In wells three and four, the fish seemed to be very unhealthy, as they weren't mobile and the majority of the fish died, besides one. The results showed that the more caffeine there was, the higher the chance of death and slow development; based on the number of embryo deaths and unhatched embryos especially in wells three and four.
**Table 1: How Many Embryo Hatched Based on Caffeine Concentration**

<table>
<thead>
<tr>
<th>Concentration of Caffeine</th>
<th>Number of Hatched Embryos</th>
<th>Number of Unhatched Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/ml</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>0.05 mg/ml</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>0.25 mg/ml</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>1 mg/ml</td>
<td>1</td>
<td>9</td>
</tr>
</tbody>
</table>

Based on the number of fish hatched, Table 1 shows that the higher concentration of caffeine, the higher chance of slow development.

**Table 2: How Many Embryo Died Based On Caffeine Concentration**

<table>
<thead>
<tr>
<th>Amount of Caffeine</th>
<th>Number of Alive Embryo</th>
<th>Number of Dead Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/ml</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>0.05 mg/ml</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>0.25 mg/ml</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>1 mg/ml</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Based on mortality, Table 2 shows that the higher concentration of caffeine, the higher chance of death.
Graph 1:

The line graph from above is based on the main idea of how many zebrafish embryos hatched depending on the amount of caffeine they were in, over the course of the experiment.

Graph 2:

The line graph from above is based from the idea of how many embryos died depending on the amount of caffeine they were tested in.
Discussion

The final results did relate to the original hypothesis, “If caffeine stunts human growth, then it may also do the same to zebrafish growth and development.” From the results, it is seen that the more caffeine the zebrafish were in, the slower they would grow. In wells one and two, the majority of the zebrafish hatched because there was very little to no caffeine affecting them. In wells three and four, only a few embryos hatched because the caffeine was affecting the growth rate, so the results do relate and support the original hypothesis. The results also related to the introduction, because the zebrafish and the fetuses ended up having a relation when being affected by caffeine. Both of them have a higher death rate when being affected by more caffeine.

A way that the lab could be improved in the future is by using a larger sample of zebrafish, and repeating the same experiment multiple times. The higher death rate in the highest concentrations of caffeine was significant when compared to lower concentrations, since less fish died in the lower concentrations.

Overall, the zebrafish had a reduced hatch rate the more concentration there was. For example, on day one, all of the embryos were still in their eggs. On day two, a few embryo hatched in wells one and two, but none in three or four. That already shows that the higher concentration is starting to affect the growth rate of the embryos. On day three, a few more in wells one and two hatched, while a couple hatched in well three and one in well four. This also supports that the hypothesis is correct, since the embryos tested in less concentration hatched a day earlier than the embryos in higher concentration. On day four, most of the embryos in wells one and two hatched while none of the embryos in wells three or four hatched, since nearly all of them died. So this does not only relate to the hypothesis, but it can also be concluded the higher concentrations of caffeine will increase the death rate for the embryos. After all, the original hypothesis can be accepted as correct. It can be inferred from this data that caffeine might have a similar effect on human embryos and should be avoided during pregnancy. This supports the literature which states that pregnant women should avoid caffeine, since it increases the chance of a miscarriage.
So in conclusion, the original hypothesis was correct. From what was learned, it can be said that higher concentrations of caffeine will cause a decreased hatch rate, and increased death rate within the zebrafish. From the results, in well four, with 1mg/ml of caffeine, all of the ten fish died, and only one hatched. In well one, with no caffeine, seven embryo hatched and only two died.

A source of error for the experiment was that no data was recorded on day two due to an absence of the student working on the project. Some suggestions to improve the experiment would be to use more fish, so the final results would be more accurate. Another one would be to also have the experiment last longer, also mainly for accuracy and more patterns or results to be discovered and recorded.

References and literature cited