The Effects of Caffeine on Zebrafish Embryos

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Abstract

The objective of this experiment was to discover how different concentrations of caffeine affects zebrafish embryos. Zebrafish embryos are a similar vertebrae to humans, therefore the results of this experiment can be akin to how caffeine effects human embryos. This research is crucial because many people consume caffeine on a regular basis. Caffeine is a common addiction in the United States and many people consume caffeine while pregnant. Researchers have found that consuming caffeine while pregnant can lower the survival rate of a fetus. This analysis was performed in order to confirm the tendencies of scientific research and expand the knowledge of how caffeine effects vertebrae fetuses. An experiment was conducted over a course of 5 days using different amounts of caffeine concentration solutions (0.00mg/mL, 0.05mg/mL, 0.25mg/mL, and 1.00mg/mL). Each day qualitative and quantitative data was collected to explain the effects of caffeine on zebrafish. The research gathered from this experiment concludes that the higher caffeine concentration is, the lower the hatch and survival rate will be.

Introduction

Caffeine is “the world's most popular drug” (Twilley, 2016). Caffeine is a natural substance commonly found in coffee beans, tea leaves, cacao seeds, and cola nut seeds. Caffeine is a stimulant that helps people stay awake and alert when consumed in small doses. People use caffeine because it is perceived to be used without harm and because caffeine is socially acceptable (Whiteman, 2015). Although caffeine can seem harmless, when ingested in large doses frequently, it can lead to anxiety, insomnia, digestive issues, muscle breakdown, addiction, high blood pressure, rapid heart rate, brain todilate, increased oxygen during sleep, nervous system problems, and fatigue (Healthline, 2019). This is why it is recommended that pregnant women do not consume caffeine. To cells, caffeine looks like adenosine, so the caffeine molecules connect to adenosine receptors mainly located in the brain. When consumed by a pregnant woman, it will go to the fetus through the placenta which it can easily pass through due to its low molecular weight and high lipid solubility (Howell, 2005). Developing fetuses metabolisms will not be able to break down caffeine as expeditiously, leaving caffeine in the fetus's developing body longer causing heart defects, lower birth weights, and miscarriages (Howell, 2005). Heart defects will last the rest of a person's life and could kill the person later on. The effects on fetuses are mainly irreversible and have to be dealt with for the rest of the person's life. If a person overdoses on caffeine, they would be given activated charcoal and have a tube inserted to wash out the contents of their stomach at a hospital. This treatment is not available to developing fetuses which is why once flourishing fetuses can overdose from caffeine and die inside a mother’s womb. Caffeine has existed for thousands of years in plants, however it was Friedlieb Ferdinand Runge, in the 1820s, who put caffeine into its purest form. Scientists began to notice the effects of caffeine around the 1850s and a century later caffeinated drinks were advertised to 2-year-olds saying that it was ‘never to early to start drinking soda.’ Scientists

found it necessary to discover the effects of caffeine on human embryos, therefore the scientists researched on a similar vertebrae, zebrafish. Zebrafish (*Danio rerio*) are an excellent alternative to human embryos considering that zebrafish embryos are clear, develop outside of the mother's body, lay a large quantities of eggs, and the eggs develop quickly (University of Oregon, 2013). To understand the effects of caffeine on humans, many people have tested the effects of caffeine on zebrafish to verify other scientist's research. For the experiment, it is predicted that the embryos exposed to the highest concentrations of caffeine will have a low hatch rate, lower survival rate, will appear under-developed, and be more energetic.

Materials and Methods

Materials

- 3 bottles of stock solutions of caffeine (0.05mg/mL, 0.25mg/mL, & 1.0 mg/mL)
- 1 beaker for dead embryos & liquid disposal
- 1 sharpie
- 1 bottle instant ocean/embryo media solution (control)
- 4 narrow pipettes
- 4 wide pipettes
- 1 multi-well plate
- 1 incubator (28.5°C)
- 1 glass slide
- 1 compound microscope
- 20 zebrafish embryos

Methods

On day one, obtain rinsed embryos. Label the well plate by obtaining a piece of masking tape and placing it on the lid of the well plate. Label the piece of tape with the caffeine concentrations; the control concentration on the control well and the experimental concentrations (0.05mg/mL, 0.25mg/mL, and 1.00mg/mL) on the experimental wells. Fill the control well on with 1 mL of Instant Ocean solution using a wide pipette. Fill the experimental wells with 1 mL of the proper caffeine concentration solutions. Make sure to put the solutions in the proper wells. Using a narrow pipette, place exactly 5 embryos in each well. Record the number of alive embryos on the data sheet (five for each section). Record any observations on the data sheet. Place the group's well plate in the incubator set at 28.5°C overnight.

On days 2, 3, and 4, remove the well plate from the incubator. Remove the dead embryos from the plate using the narrow pipette and squirt them in the waste beaker for dead embryos and the previous day's solutions. Be careful to only remove the dead embryos. Count the remaining embryos and any hatched fish and record the data in the quantitative data table. Observe the

embryos under the light microscope and the stereoscope to observe any abnormal movement, deformities, behaviors, developmental rates, or survival rates. Record this data in the qualitative data sheet. Remove solutions from the wells. It is easiest to tilt the plate so the embryos settle to the bottom and then remove the liquid from the top of the well cavity. Replace the previous days solutions with fresh solutions using a clean pipette. Do not cross contaminate when using pipettes and beakers, use a new clean pipette for each solution concentration. Return the well plate to the incubator.

On day five, remove the well plate from the incubator. Remove the dead embryos from the well plate as discussed above. Count the remaining embryos and any hatched fish and record quantitative data in the correct data table. Place the plate under the microscopes and record qualitative observations on the data sheet. Place all embryos and hatched fish in the waste container. Properly dispose of the organisms.

Safety Precautions: Wear goggles at all times to prevent any chemicals or substances getting into someone's eyes. Wear gloves when dealing with chemicals to prevent any harm to the body.

Results

This experiment was conducted in order to learn about the effects of caffeine on zebrafish embryos and how those effects transcribe to the effects of consuming caffeine while a woman is pregnant. It was hypothesized that the embryos exposed to the highest concentrations of caffeine will have a low hatch rate, lower survival rate, will appear under-developed, and be more energetic. The dependent variable of this experiment was the concentration of caffeine which included the control well with 0.00mg/mL, and 3 experimental wells with 0.05mg/mL, 0.25mg/mL, and 1.0mg/mL caffeine concentration in each. The independent variables were the survival rate and the hatch rate of embryos. Experimental constants included the wells, the same amount of solution in each individual well cavity, the number of embryos collected at the beginning of the experiment, and the temperature of the incubator. The general pattern of our data is that the less caffeine zebrafish embryos were exposed to, the higher their survival and hatch rate was. As seen in figure 1, all of the embryos in experimental well #3 with 1.00mg/mL concentration caffeine solution died after day 2. While in experimental well #1 with 0.05mg/mL concentration caffeine solution, majority of the embryos lived until the fourth day. The same results can be told for the hatched vs. unhatched data. Figure 3 shows that no embryos from experimental wells #3 and #1 hatched and that one embryo from experimental well #2 hatched but died the following day. The control well was the only well to contain any survived and hatched embryos that lasted the entirety of the experiment. The results show that on day 2, the zebrafish started to die, in the control well, as one of the four of the zebrafish embryos died. The zebrafish continued to die slowly each day of the experiment, commonly in the wells holding high concentrations of caffeine solution.

Table 1:

Zebrafish Embryos Alive vs Dead at Different Concentrations of Caffeine Over One Week					
Time	hpf	Control Group (well #1) Alive / Dead	Experimental Group Concentration 0.05mg/mL (well #2) Alive / Dead	Experimental Group Concentration 0.25mg/mL (well #3) Alive / Dead	Experimental Group Concentration 1.0mg/mL (well #4) Alive / Dead
Day 1	0 hpf	5/0	5/0	5/0	5/0
Day 2	24 hpf	4/1	4/1	4/1	4/1
Day 3	48 hpf	3/1	4/0	3/1	0/4
Day 4	72 hpf	1/2	4/0	2/1	0/0
Day 5	96 hpf	1/0	0/4	0/2	0/0

The table above shows the results of the zebrafish alive and dead over the course of one week with the different amounts of caffeine concentration; 0.00mg/mL, 0.05mg/mL,0.25mg/mL, and 1.0mg/mL.

Table 2:

Zebrafish Embryos Hatched vs Unhatched at Different Concentrations of Caffeine Over One Week					
Time	hpf	Control Group (well #1) H / U	Experimental Group Concentration 0.05mg/mL (well #2) H / U	Experimental Group Concentration 0.25mg/mL (well #3) H / U	Experimental Group Concentration 1.0mg/mL (well #4) H / U
Day 1	0 hpf	0 / 5	0 / 5	0/5	0/5
Day 2	24 hpf	0/4	0/4	0/4	0/4
Day 3	48 hpf	0/3	0/2	0/3	0/0
Day 4	72 hpf	1/0	0/1	1/1	0/0
Day 5	96 hpf	1/0	0/0	0/0	0/0

The table overhead shows the results of the zebrafish embryos hatched and unhatched over the course of one week with the different amounts of caffeine concentration; 0.00mg/mL, 0.05mg/mL,0.25mg/mL, and 1.0mg/mL.

Figure 1:

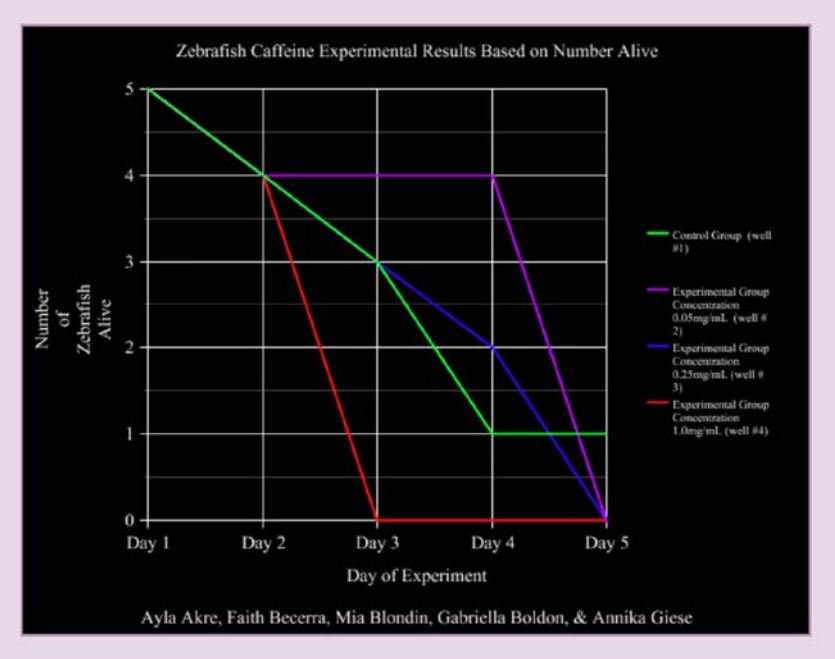

Figure 1 is a line graph showing the results of the numbers of zebrafish alive over the course of one week with the different amounts of caffeine concentration; 0.00mg/mL, 0.05mg/mL,0.25mg/mL, and 1.0mg/mL.

Figure 2:

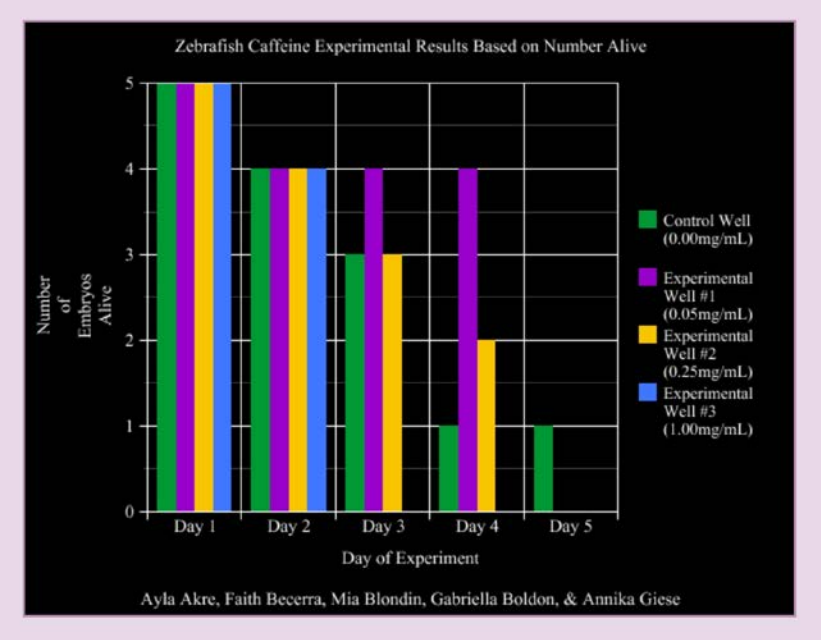

Figure 2 is a bar graph showing the number of embryos alive over the course of one week with the different amounts of caffeine concentration; 0.00mg/mL, 0.05mg/mL,0.25mg/mL, and 1.0mg/mL.

Figure 3:

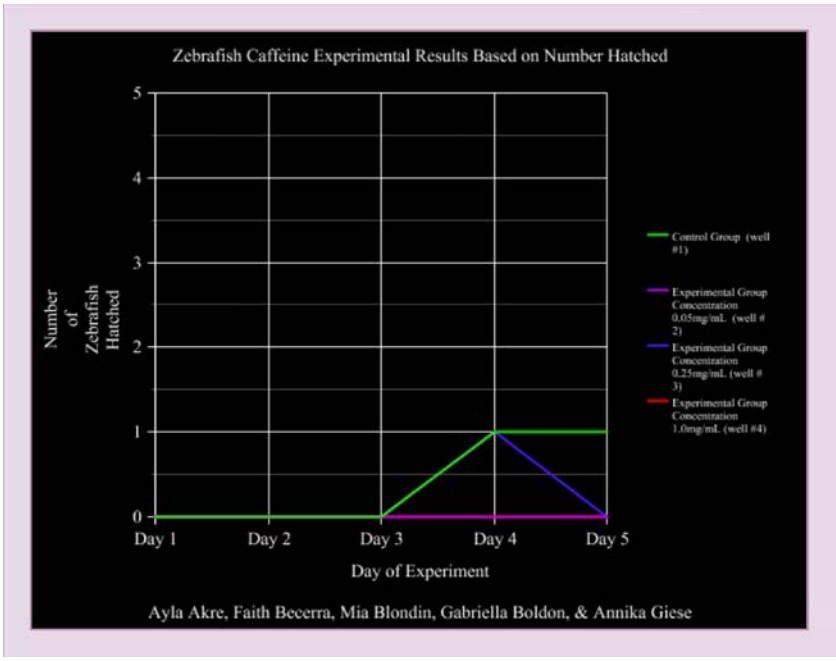

Figure 3 is a line graph showing the results of the number of zebrafish hatched over the course of one week with the different amounts of caffeine concentration; 0.00mg/mL, 0.05mg/mL,0.25mg/mL, and 1.0mg/mL.

Figure 4:

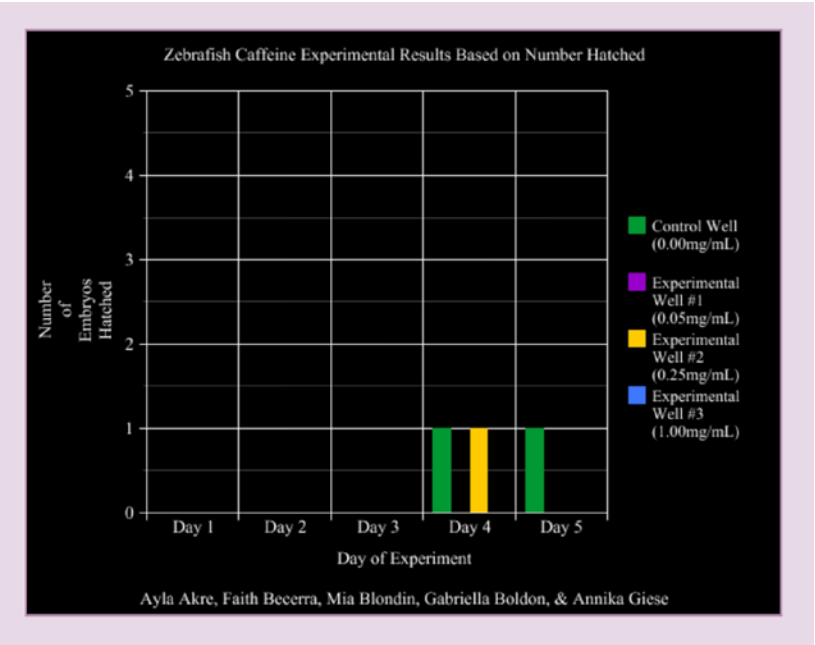

Figure 4 is a bar graph showing the results of the number of embryos hatched over the course of one week with the different amounts of caffeine concentration; 0.00mg/mL, 0.05mg/mL,0.25mg/mL, and 1.0mg/mL.

Figure 5:

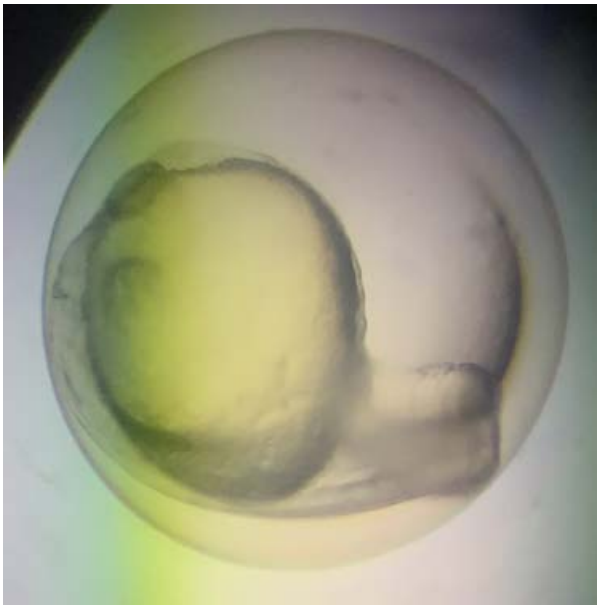

Figure 5 illustrates a zebrafish embryo from the control well on day 2.

Figure 6:

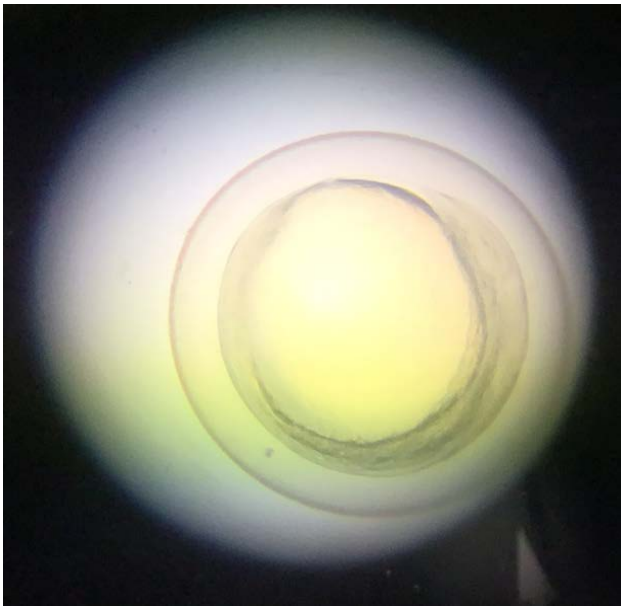

Figure 6 portrays a zebrafish embryo on day 2 from experimental well #1 containing 0.05mg/mL concentration caffeine solution.

Figure 7:

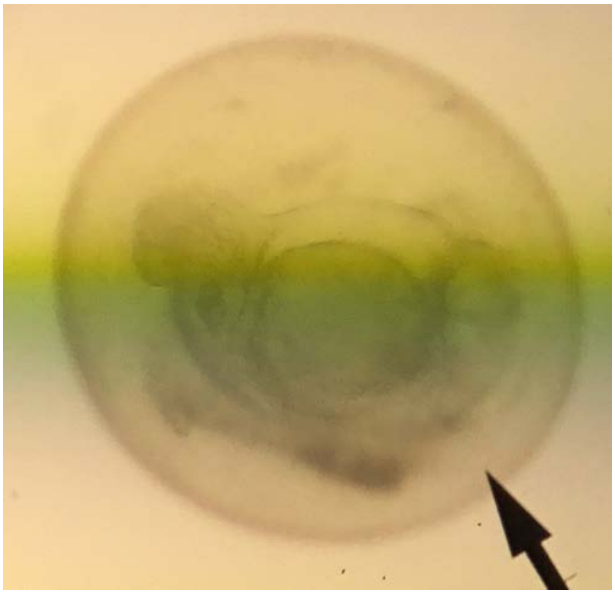

Figure 7 shows a zebrafish embryo on day 2 in experimental well #2 with 0.25mg/mL caffeine concentration.

Figure 8:

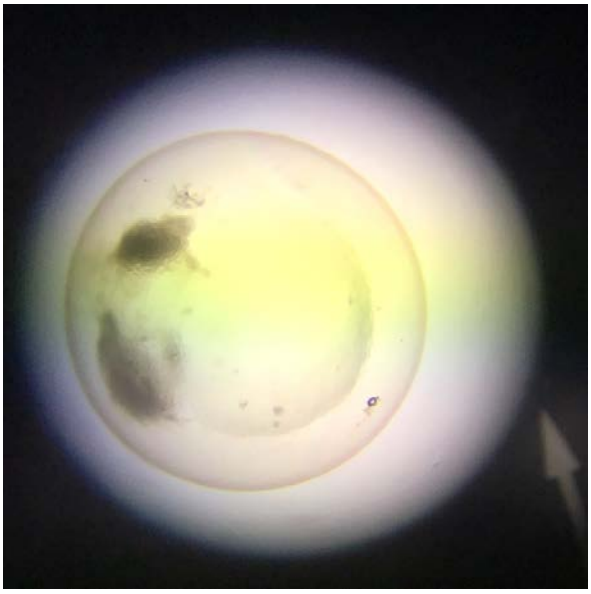

Figure 8 displays a zebrafish embryo on day 2 from experimental well #3 (1.00mg/mL).

Figure 9:

Figure 9 depicts a hatched zebrafish from the control well on day 5.

Discussion

The hypothesis for this experiment was proven to be true because it was stated that the embryos exposed to the highest concentrations of caffeine would have a low hatch rate, lower survival rate, will appear under-developed, and be energetic. For example, on day 3, in experimental well #3 (the highest concentration group with 1.00mg/mL caffeine solution) four out of four of the embryos had died which can be seen in figure 1 of the results section. The embryos also appeared to be underdeveloped as they were smaller in stature and slightly deformed as can be seen in figure 8. The zebrafish in experimental wells #1 and #2 also appeared to be more energetic and had more movement than the embryos in the control well. Although this experiment has explained much about the effects of caffeine on zebrafish, there are some limitations to this experiment such as time and the number of organism tested upon. If more time were provided, it would give a more accurate result as to observe how the fish developed into an adult to observe how the effects of caffeine on zebrafish embryos can translate to adulthood in humans. If provided more embryos, more accurate results would be in place. This is because the more specimens an experiment is performed upon, the more accurate the data will be due to the fact that a larger scale general trend will be made. Although this experiment was performed following lab instructions, there may be some errors in our data. It is unclear as to the way zebrafish embryos looked on day 3, because of the fact that no pictures were taken of them. This experiment is best performed throughout the duration of two weeks with twice as many zebrafish and that the results of the alive, dead, hatched, and unhatched data is collected everyday. As stated earlier, this experiment was performed to the highest capabilities and although some

results such as the large number of zebrafish embryos alive in experimental well #2 are seen as trivial results this could also explain how small amounts caffeine can be beneficial to human embryos. The purpose of this experiment was to discover the effects of caffeine on zebrafish embryos to foresee the effects caffeine could have on human embryos by using a comparative vertebrate to humans, zebrafish. The control well with no caffeine concentration was the only well to successfully keep an embryo alive and hatched over the course of 5 days. As shown in figure 2, experimental wells #1 and #2 with lower amounts of caffeine concentration were able to keep the embryos alive and some embryos hatched for 4 days. In experimental well #3 with the highest concentration (1.00mg/mL), all of the zebrafish embryos died within 2 days. This data shows that not consuming caffeine is the best way to ensure a healthy baby, but it is possible to have a healthy baby with lower amounts of caffeine as well. A healthy baby will acquire normal growth and development in its life and will not have to deal with any limitations in their future due to the caffeine consumption of their mother during pregnancy.

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