Development of Zebrafish Embryos in Caffeine Solution

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Abstract

In the following experiment, zebrafish embryos were exposed to caffeine in order to test the effects of the substance on their development. The embryos were placed in two different concentrations of caffeine (.05 mg/mL and .25 mg/mL) as well as a control concentration in order to compare the results. The zebrafish embryos were observed and counted for the number of embryos alive and hatched at 24, 48, and 72 hours post-fertilization. Numerical results showed a slight trend towards decreased hatch numbers as caffeine concentration increased. While altogether no data was considered statistically significant by an unpaired t-test, qualitative data showed that zebrafish embryos exposed to the caffeine solutions tended to show slowed or incomplete development and premature hatching. Zebrafish that did hatch were visibly shorter in length and showed cases of swim bladder. Results from this experiment showed the possibility of a connection between poor embryonic development and caffeine exposure. This applies to human health because pregnant women that consume caffeine may adversely affect the fetus in the womb by hindering its development.

Introduction

Coffee is the most consumed drink in the world. It is a valuable part of the world economy, with an average world exportation rate of 90 million bags of 60 kg each per year, and in 2008, world coffee consumption reached 128 million bags for the year.[1] With so many people drinking the beverage, it is important to know the contents-- caffeine being the most well-known. Arabica coffee can contain between .8 to 1.4% caffeine, while Robusta coffee can have 1.7 to 4.0%.[1]

Caffeine itself has been associated with varying health effects. Intake has been connected to increased blood pressure and higher plasma concentrations of cholesterol and homocysteine (an amino acid in the blood linked to heart disease).[2] High doses of caffeine could result in symptoms related to the cardiovascular, central nervous, and gastrointestinal systems.[3] Moreover, children that regularly consume caffeine tended to experience decreased reaction times.[4] For women, coffee drinking was associated with a higher risk of fracture. More prominently, however, pregnant women experienced a greater number of preterm births in both the first and second trimesters, and high coffee consumption was also linked to low birth weight.[5]

Instead of using human embryos to test the effects of caffeine in this experiment, zebrafish embryos will mature in two different concentrations of caffeine, including .05 mg/mL and .25 mg/mL, and a control concentration will be used to compare the results. Zebrafish make excellent substitutions for human embryos for several reasons. First, the fish have transparent eggs, which allows for observation of the development of the embryo.[6] Furthermore, the nervous systems of zebrafish and humans have similar pathways, and both species demonstrate similar social, emotional, and psychological behaviors. Due to their simple natural habitat, zebrafish are easy and cost-efficient to maintain in a laboratory. With short generations

of 3 to 5 months and high egg production, they make perfect candidates for embryo experimentation.[7]

This experiment aims to determine the effects that caffeine might have on developing embryos, using zebrafish embryos as a replacement for human embryos. That is, will zebrafish embryo development be affected by exposure to caffeine? Because caffeine has a half-life that is on average 8.3 hours longer in pregnant women, the effects of caffeine will last longer in fetuses.[8] Caffeine also has a longer half-life in newborn infants and lasts a prolonged amount of time, similar to caffeine in fetuses.[9] Due to this, it is possible that the usual neurological impairments caused by the effects of caffeine may be magnified in the zebrafish embryos.

Additionally, experiments conducted with zebrafish embryonic caffeine exposure have shown physical differences between control and experimental groups, such as shortened body length and misaligned muscle fibers. Neurologically, the experimental zebrafish also displayed a noticeable reduction in tactile sensitivity and defects in the motor neurons.[6] Under similar experimental conditions, it is likely that these differences in physical and psychological makeup may repeat themselves, and the same results may be apparent in this zebrafish experiment.

Materials and Methods

The following procedure and materials are modified from SEPA UW-Milwaukee.

Materials

1 Well plate
120 Zebrafish embryos
Waste beaker
1 Bottle of Instant Ocean/Embryo Media Solution
1 Bottle of each caffeine solution: .05, .25 (as recommended by UW-Milwaukee)
1 Bottle of methyl blue solution
12 Disposable pipettes
Masking Tape
Sharpie
Incubator (28.5℃)
Compound microscope and slide

Procedure

Day 1

a. Obtain rinsed embryos.
b. Label plate with name and class hour. Label the caffeine concentration of each well using the sharpie and tape provided.
c. Fill four control wells of the plate with 1 mL of Instant Ocean/Embryo Media solution using the disposable pipette. Fill four wells with .25 caffeine solution, and the remaining wells with .05 caffeine solution. Divide the embryos so there are approximately 10 embryos in each well.
d. Record the number of live starting embryos on data table.
e. Observe under the dissecting microscope. Take pictures and record observations on data table.

f. Place the well plate into 28.5℃ incubator for 24 hours.

Day 2

a. Remove well plate from the incubator.
b. Remove the dead embryos from the plate using the disposable pipettes. Remember to use a new pipette for each different caffeine concentration. Squirt dead embryos into waste beaker.
c. Count remaining embryos in the wells, as well as hatched fish, and record these numbers in the data table.
d. Remove the liquid solutions from each well of the plate.
e. Replace the caffeine solutions with the appropriate fresh caffeine solution using a clean pipette for each concentration.
f. Place well plate under dissecting microscope and record observations. Note and take pictures of any abnormalities or developmental markers.
g. Return the well plate to the incubator.

Day 3

a. Repeat day 2 work and observations. Record and take pictures of all data.
b. Place all embryos and fish in a waste container.

Data

Data for this experiment was collected from several other groups in order to optimize accuracy.

Data Table 1: Control Solution

Time after Fertilization (hours)	0 Hours Post-Fertilization	24 Hours Post-Fertilization	48 Hours Post-Fertilization	72 Hours Post-Fertilization
# Fish Hatched	0	0	18	25
# Fish Alive	40	32	32	32

Data Table 2: .05 mg/mL Solution

Time after Fertilization (hours)	0 Hours Post-Fertilization	24 Hours Post-Fertilization	48 Hours Post-Fertilization	72 Hours Post-Fertilization
# Fish Hatched	0	0	8	23
# Fish Alive	40	30	30	30

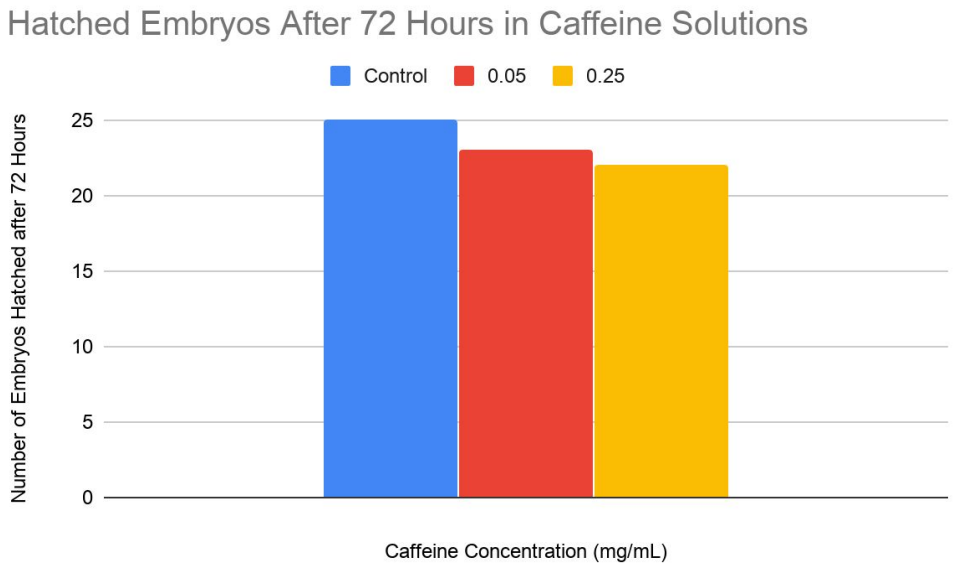
Data Table 3: .25 mg/mL Solution

Time after Fertilization (hours)	0 Hours Post-Fertilization	24 Hours Post-Fertilization	48 Hours Post-Fertilization	72 Hours Post-Fertilization
# Fish Hatched	0	0	20	22
# Fish Alive	40	31	31	30

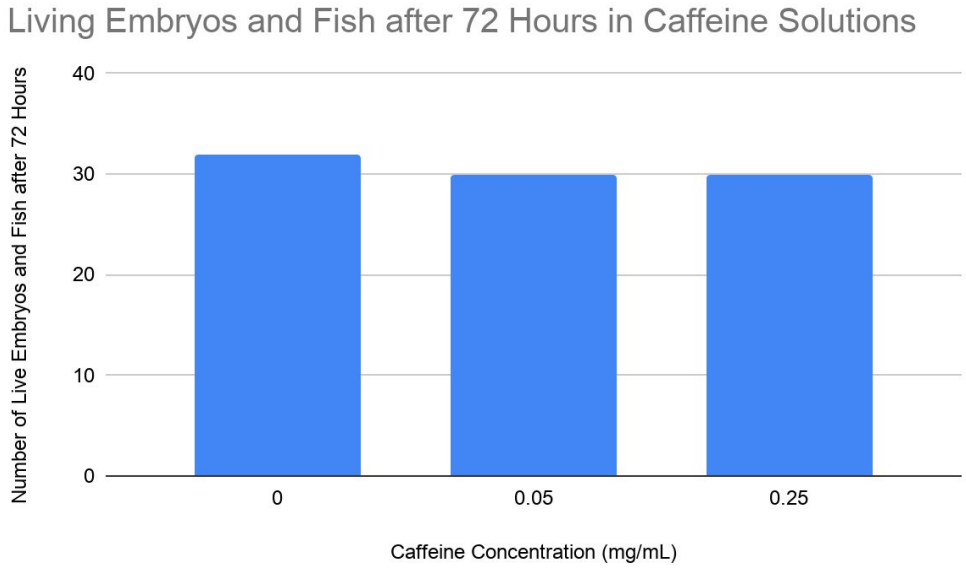
Data Tables 1-3 reflect the quantitative values recorded for the number of live fish and number of hatched fish for each 24-hour period after the embryos have been fertilized. Data Table 1

contains the recorded number of live and hatched fish for the control solution (0 mg/mL Caffeine). Meanwhile Data Tables 2 and 3 each contain the number of live and hatched fish for .05 mg/mL caffeine solution and .25 mg/mL caffeine solution respectively.

Graph 1



Graph 2



Graphs 1-2 compare the effects of each caffeine concentration after 72 hours. In graph 1, for example, the number of hatched embryos was compared among all 3 tested caffeine solutions. Visibly, the number of hatched fish decreased the higher the concentration. Similarly, Graph 2 compares the number of live embryos and fish after 72 hours among the 3 caffeine solution concentrations. While the correlation is not as obvious, again the number appears to decrease as concentration increases.

Figure 1

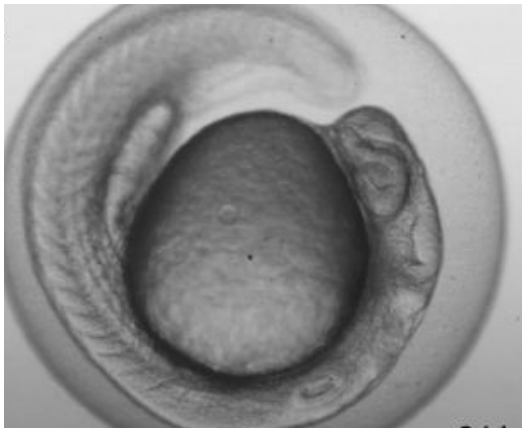


Figure 2

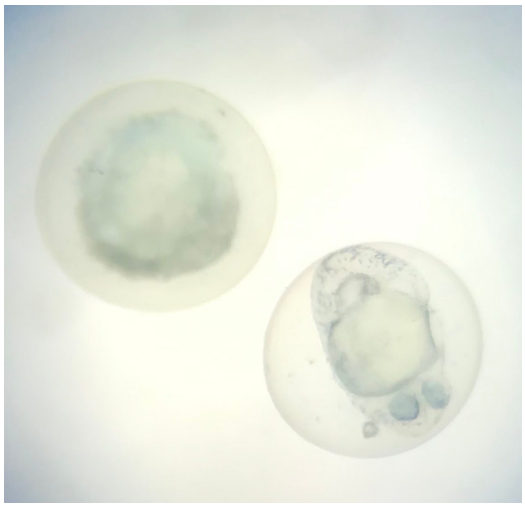


Figure 3

Figure 1 shows what a healthy zebrafish embryo should look like after 24 hours. This may be useful to compare to figures 2-3. Image obtained from a publication.[10]

Figure 2 is a photo taken of a zebrafish embryo in .25 mg/mL caffeine solution. It was taken 24 hours post-fertilization. In the photo, a decomposing embryo (left) is shown next to an embryo that is behind in development (right). The zebrafish embryo shown at right in the picture appears to have dark, developed eyes, but the embryo's body lacks the same defined development shown in the eyes.

Figure 3 is a photo taken of a zebrafish embryo in .05 mg/mL caffeine solution after 24 hours. The darkened gray areas in the image indicate that the embryo is already decomposing. The embryo is dead, and no developmental markers are visible.

Data Analysis

In order to determine the significance of the numerical results of this experiment, an unpaired t-test compared the control group's number of hatched and alive embryos to the numbers from the two test group concentrations. To do this, the t-test compared the mean of the control group with the mean of each experimental group to find a P-value, or the probability that the results were caused by mere chance. In this case, all P-values were much greater than .05, which is the number that a P-value must be lower than in order to be considered statistically significant. Every experimental data set for this experiment therefore yielded no statistically significant data. This means that the hypothesis is not supported.

Results

This experiment was designed to test and observe the effects of caffeine exposure to zebrafish embryos by placing the embryos in varying concentration levels of caffeine solution. The number of live and hatched embryos was recorded and compared among the control concentration, .05 mg/mL, and .25 mg/mL concentrations. Observations were also made regarding abnormalities in the experimental groups.

In this experiment, the independent variable was the caffeine concentration that the fish were exposed to, while the dependent variables were the number of live fish, the number of hatched fish, and the deformations that resulted from caffeine exposure. The caffeine concentration of each test group was to affect how embryos lived, hatched, or mutated in comparison with the control group. The control group was a group of embryos not exposed to any caffeine in order to determine what "normal" embryo development may look like.

While this particular experiment did not draw fully conclusive results regarding significant numerical data, some graphs showed there may possibly be a trend between live and hatched zebrafish and the caffeine concentration they are exposed to. Beyond numerical data, however, it is clear that caffeine does have some effect on the ability for zebrafish embryos to completely develop. The aims of this experiment were therefore carried out because the effects of caffeine were observed and suggested a connection between caffeine exposure and developmental ability.

Discussion

While the numerical results from this experiment were not statistically significant, qualitative data showed some effects of the caffeine on the zebrafish embryos. Embryos in the .05 mg/mL and .25 mg/mL both displayed slowed development and inability for the fish body to grow at the same rate as the eyes and other parts of the body. Fish that hatched often showed these mutations, as most-- if not all-- hatched prematurely, their bodies not yet ready to emerge from the embryo. Those that did hatch successfully tended to have shorter body length, which also indicated delayed development in the body. The fish also had stouter bodies and enlarged, extended swim bladder. Graphs shown in the data reflected the decreased hatching among embryos exposed to caffeine. Despite this, because the data gathered is not considered statistically significant, the hypothesis failed to be supported.

A few limitations in this experiment may have prevented statistically significant findings. For one, a low number of embryos used in the experiment limited the statistical accuracy of the data, and in larger experiments it would be easier to gather significant data by using a larger sample. Other limitations might include the guaranteed health of the embryos, as it was not possible in this experiment to determine the absolute initial health of the embryos. If they were already unhealthy or unsanitary, it could change the outcome of the experiment.

Despite these limitations and the lack of statistical significance, however, important qualitative data from this experiment suggests that there may be a connection between poor embryo development and exposure to caffeine. As part of the hypothesis, the fish were expected to have magnified neurological impairments, as caffeine has a longer half-life in fetuses (or embryos).[8] While this was not evident, other changes in the zebrafish were. It was predicted in the hypothesis that the fish would show shortened body length and misaligned muscle fibers.[6] Indeed, the fish exposed to caffeine in this experiment were visibly shorter in length with stouter bodies.

Ultimately, this experiment and research is important to human health. Caffeine is consumed across the world, especially in beverages like coffee, for example.[1] This experiment tested how zebrafish embryos might be affected by exposure to caffeine. In turn, the results from this experiment may indicate how human fetuses may react to caffeine exposure inside the womb of pregnant women that consume caffeinated beverages.

Resources

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