The Effects Caffeine Has On the Development of a Zebrafish Embryo

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Abstract

In this lab, we tested the effects of caffeine on developing zebrafish embryos, specifically a zebrafish. Because the development of zebrafish and humans are in many ways similar, our results help us understand the many effects caffeine has on the growth of a human embryo. We found that the higher the concentration of caffeine, the greater chances of birth defects and fatality there would be. The zebrafish embryos with little to no caffeine had the best odds of survival and no birth defects. With these results, it is advised that expecting mothers should limit the amount of caffeine they have during the developmental process of the child.

Introduction

In this lab, we are testing whether or not caffeine will have an effect on the development of a zebrafish embryo. The information we find will also tell us what effects caffeine has on the development of a human baby. This is because 70% of human genes can be found in a zebrafishes genetic code, too (Tiny).

Zebrafish serve as good models for human development because they pass from the egg to the larval stage in less than three days and reproduce very easily. Along with that, the transparent skin of the zebrafish embryo allows biologists to easily see any changes that have occurred during development (Zebrafish).

For expecting mothers, it is advised that they should limit their caffeine input to about 200 milligrams a day, which is equivalent to one tall cup of coffee or five shots of espresso (Caffeine Safe). When a pregnant woman has a caffeinated drink, that caffeine travels through the placenta straight into the baby’s bloodstream. Although the mother's metabolism does get rid of the caffeine fairly quickly, it takes longer for the child to do the same. Mild exposure to caffeine won’t hurt the baby, however studies have shown that “mothers who consumed more

than 300 mg of caffeine a day were more likely to give birth to babies who were small for their age (Caffeine during).” Also, large amounts of caffeine could result in several birth defects and possibly a miscarriage (Caffeine intake). With that said, we hypothesize that if large amounts of caffeine are taken in during the development of a zebrafish embryo, then there will be a greater chance of birth defects, a smaller survival rate, and a high chance of the zebrafish being small for its age because caffeine has been scientifically proven to affect the development of embryos.

Materials

- 1 dissecting microscope
- 1 12 well plate
- Instant ocean solution
- Small amount of methylene blue solution
- .05 mg/ml, .25 mg/ml, and 1 mg/ml of caffeine
- 120 zebrafish eggs
- 6 pipets each day (3 small for caffeine, 1 small for instant ocean, 1 small for methylene blue, and 1 large for eggs)
- Safety goggles
- 1 Sharpie

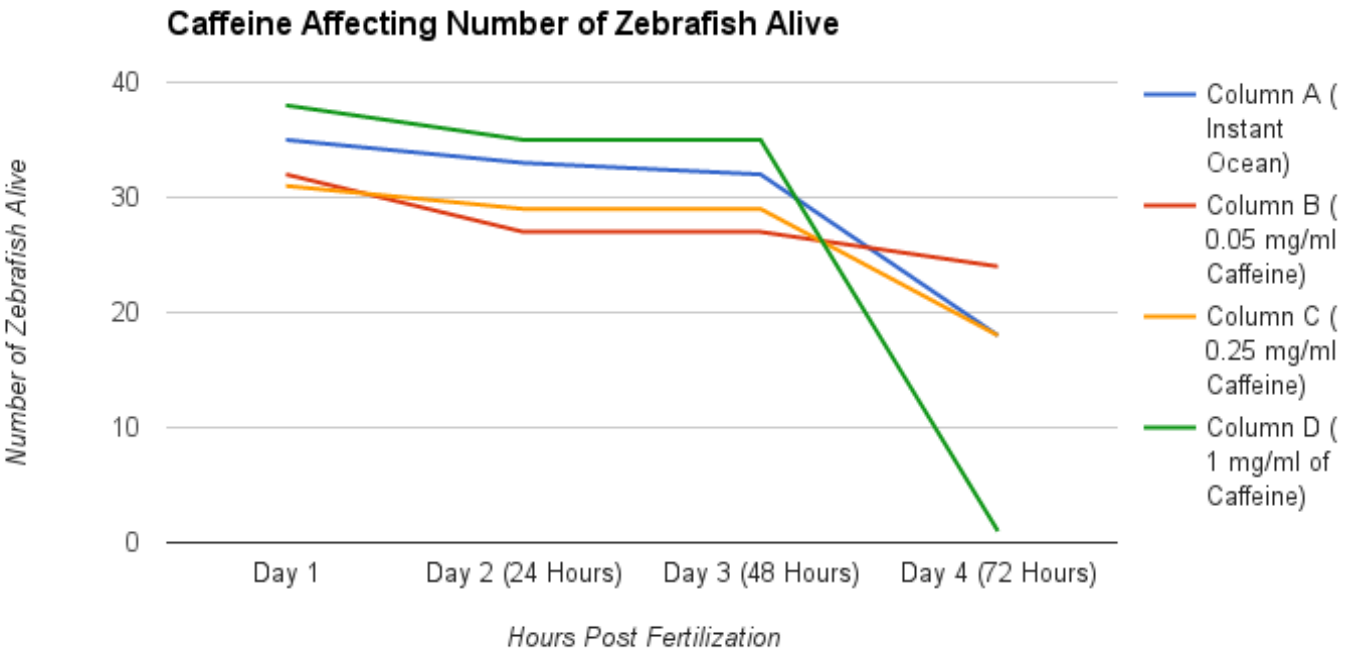
Methods

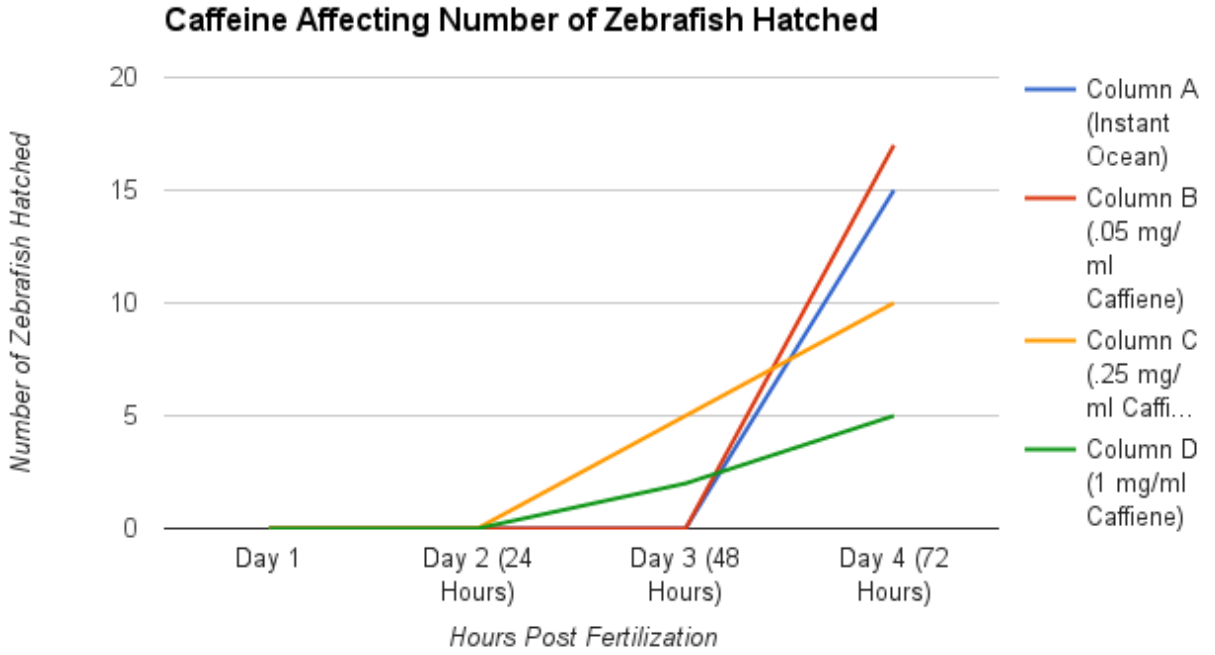
1. Gather materials and put safety goggles on.
2. Label the 12 well plate (columns: A/B/C/D and rows: 1/2/3) with a sharpie.
3. Using a large pipet, add ten zebrafish eggs to each individual well.

4. Add 2 ml of instant ocean to each well in Column A with a small pipet.
5. Add 2 ml of caffeine to only columns B, C, and D. (No caffeine is needed in column A because it is the control group.) Make sure to use a new pipet for each column to prevent cross-contamination.
    a. Insert 2 ml of .05 mg/ml caffeine to the wells in column B
    b. Insert 2 ml of .25 mg/ml caffeine to the wells in column C
    c. Insert 2 ml of 1 mg/ml caffeine to the wells in column D
6. Dip a small pipet into the methylene blue. Add a drop to every well. Start with column A, and then move to B, and so on. This will prevent cross contamination.
7. Place well plate in an incubator at 28.5°C.
8. Days 2-4:
    a. Start by removing almost all of the liquids and any dead eggs in each well with a large pipet. Begin with column A, and go alphabetically from there.
    b. Record how many eggs are hatched or alive, along with the physical conditions of the eggs. Add this information into a chart like the one below. (Optional: take pictures through the microscope with camera to record progress).
    c. Repeat steps 4 - 6.
    d. Place well plate in an incubator at 28.5°C.

Numbers of Zebrafish Alive and Hatched Over Time

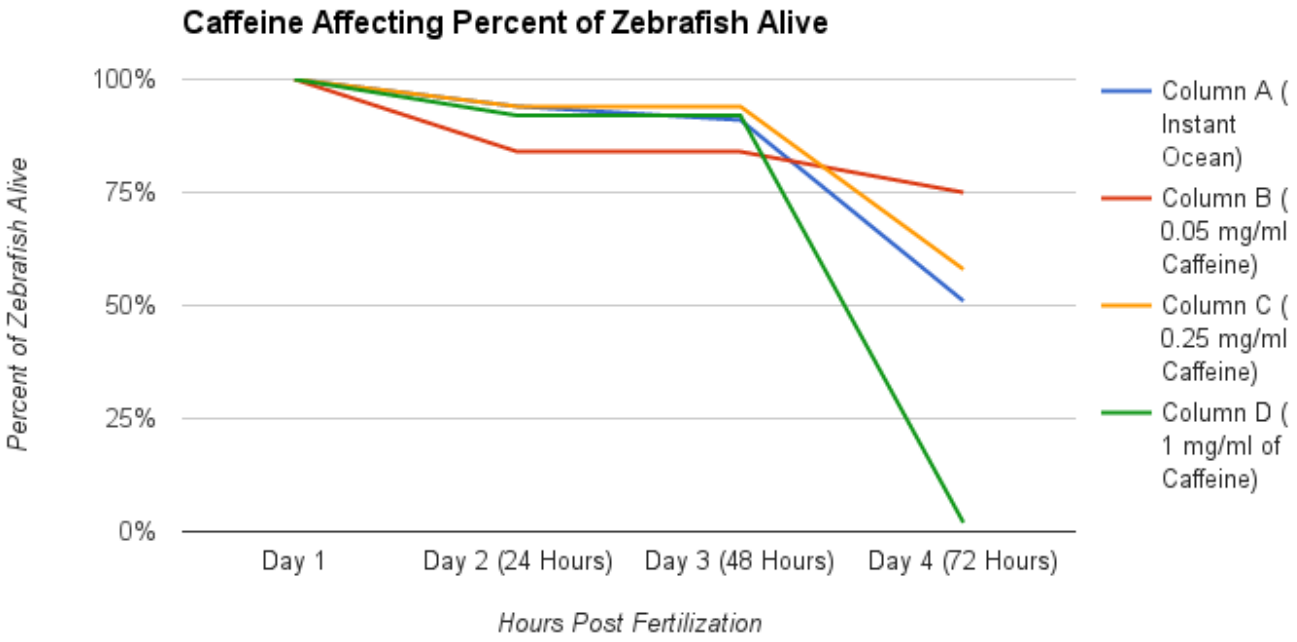
Treatment	Well #	# of starting fish	24 hours post fertilization (Day 2)	48 hours post fertilization (Day 3)	72 hours post fertilization (Day 4)
			#hatched/#live	#hatched/#live	#hatched/#live
Instant Ocean	A1	13	0/12	0/11	0/0
Instant Ocean	A2	10	0/9	0/9	6/8
Instant Ocean	A3	12	0/12	0/12	9/10
0.05 mg/ml of Caffeine	B1	8	0/6	0/6	6/6
0.05 mg/ml of Caffeine	B2	10	0/9	0/9	0/6
0.05 mg/l of Caffeine	B3	14	0/12	0/12	10/12
0.25 mg/ml of Caffeine	C1	10	0/9	3/9	7/8
0.25 mg/ml of Caffeine	C2	11	0/10	0/10	0/0
0.25 mg/ml of Caffeine	C3	10	0/10	2/10	3/10
1 mg/ml of Caffeine	D1	15	0/13	1/13	1/0
1 mg/ml of Caffeine	D2	10	0/9	1/9	1/0
1 mg/ml of Caffeine	D3	13	0/13	0/10	1/1

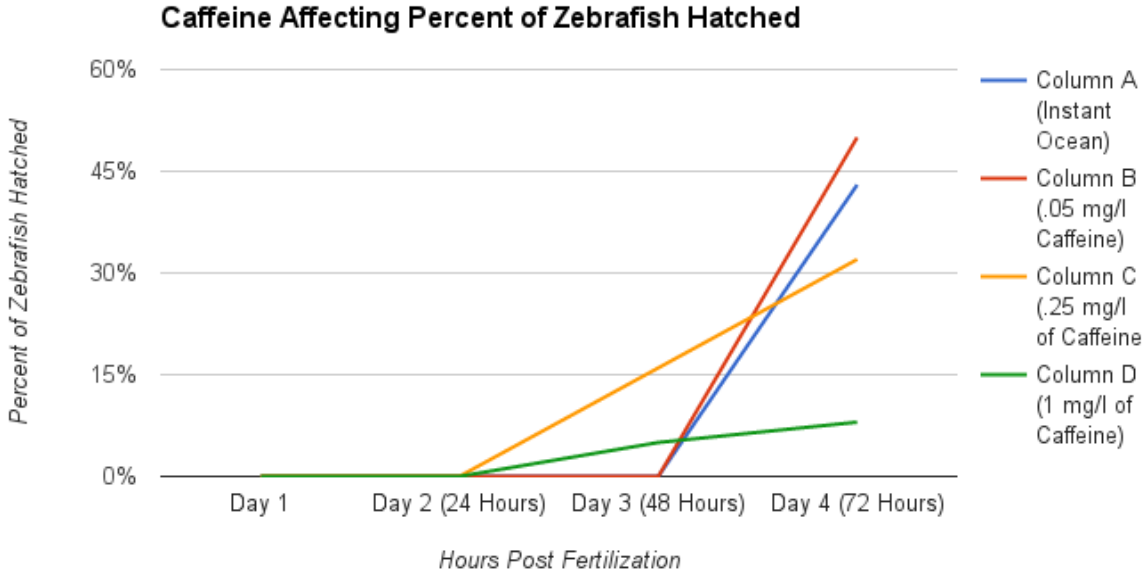

Caffeine Affecting Number of Zebrafish Alive
Number of Zebrafish Alive
40
30
20
10
0
Day 1
Day 2 (24 Hours)
Day 3 (48 Hours)
Day 4 (72 Hours)
Hours Post Fertilization
Column A (Instant Ocean)
Column B (0.05 mg/ml Caffeine)
Column C (0.25 mg/ml Caffeine)
Column D (1 mg/ml of Caffeine)


Caffeine Affecting Number of Zebrafish Hatched
Number of Zebrafish Hatched
20
15
10
5
0
Day 1
Day 2 (24 Hours)
Day 3 (48 Hours)
Day 4 (72 Hours)
Hours Post Fertilization
Column A (Instant Ocean)
Column B (.05 mg/ml Caffiene)
Column C (.25 mg/ml Caffi...
Column D (1 mg/ml Caffiene)

Percents of Zebrafish Alive and Hatched Over Time

Treatment	Well #	# of starting fish	24 hours post fertilization (Day 2)	48 hours post fertilization (Day 3)	72 hours post fertilization (Day 4)
			#hatched/#live	#hatched/#live	#hatched/#live
Instant Ocean	A1	13	0%/92%	0%/85%	0%/0%
Instant Ocean	A2	10	0%/90%	0%/90%	60%/80%
Instant Ocean	A3	12	0%/100%	0%/100%	75%/83%
0.05 mg/ml of Caffeine	B1	8	0%/75%	0%/75%	75%/75%
0.05 mg/ml of Caffeine	B2	10	0%/90%	0%/90%	0%/60%
0.05 mg/l of Caffeine	B3	14	0%/86%	0%/86%	71%/86%
0.25 mg/ml of Caffeine	C1	10	0%/90%	30%/100%	70%/80%
0.25 mg/ml of Caffeine	C2	11	0%/91%	0%/91%	0%/0%
0.25 mg/ml of Caffeine	C3	10	0%/100%	20%/100%	30%/100%
1 mg/ml of Caffeine	D1	15	0%/87%	7%/87%	7%/0%
1 mg/ml of Caffeine	D2	10	0%/90%	10%/90%	10%/0%
1 mg/ml of Caffeine	D3	13	0%/100%	0%/77%	8%/8%


Caffeine Affecting Percent of Zebrafish Alive
100%
75%
50%
25%
0%
Percent of Zebrafish Alive
Day 1
Day 2 (24 Hours)
Day 3 (48 Hours)
Day 4 (72 Hours)
Hours Post Fertilization
Column A (Instant Ocean)
Column B (0.05 mg/ml Caffeine)
Column C (0.25 mg/ml Caffeine)
Column D (1 mg/ml of Caffeine)


Caffeine Affecting Percent of Zebrafish Hatched
60%
45%
30%
15%
0%
Percent of Zebrafish Hatched
Day 1
Day 2 (24 Hours)
Day 3 (48 Hours)
Day 4 (72 Hours)
Hours Post Fertilization
Column A (Instant Ocean)
Column B (.05 mg/l Caffeine)
Column C (.25 mg/l of Caffeine
Column D (1 mg/l of Caffeine)

Results

In this lab, our independent variable was the amount of caffeine added to each well, and the dependent variable was the condition of the zebrafish - whether they were alive, hatched, or had birth defects. The control group in the lab was column A, the group with no caffeine and only instant ocean. Columns B-D were our experimental groups and each had different amounts of caffeine in them. Column B had .05 mg/ml of caffeine, column C had .25 mg/ml of caffeine, and column D had 1 mg/ml of caffeine in each well.

Initially over the first 3 days, all four groups had a similar decrease in the survival rate of zebrafish. Column D had the most dramatic drop in survival rate between day 3 and 4, going from 85% to 3% of alive fish (see figure 3). Towards the beginning of the lab, the eggs in the columns with more caffeine (columns C and D) were the first to start hatching. However, by the last day, the columns with little to no caffeine (columns A and B) had the most amount of hatched eggs. Most of the hatched eggs in columns C-D had curved spines by day 3 (see figure 1). For the most part, the hatched eggs in columns A and B had almost no birth defects and a very low amount of dead eggs by day 4. The majority of eggs in these groups had healthy, straight backs (see figure 2). In conclusion, if a low amount of caffeine was added, there would be a greater hatch rate and lesser chance of birth defects by the final day.

Figure 1: The circled zebrafish are examples of curved spines, commonly found in columns C and D where the caffeine concentration was the highest.

Figure 2: The zebrafish that are circled have healthy, straight spines. These zebrafish were mainly found in columns A and B, and occasionally C, where the caffeine concentration was fairly low.

Figure 3: The picture above shows a dead zebrafish egg that had not hatched in well D3. This happened often in column D towards the end of the lab.

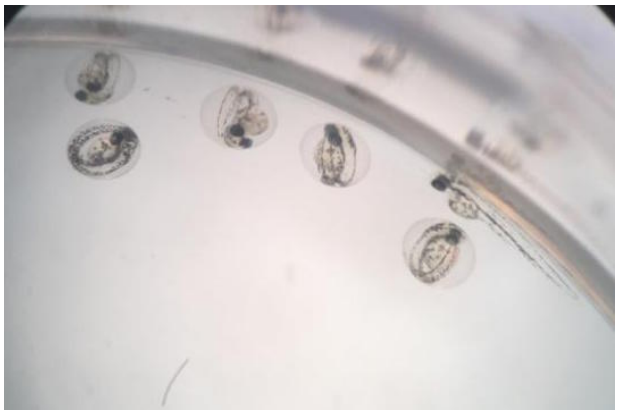


Figure 4: This picture was taken of unhatched, but alive, zebrafish in column C.

Discussion

Our hypothesis was that the more caffeine that was added to a well, the lower chance of survival and higher chance of birth defects there would be. The lab we conducted proved our hypothesis correct. In our experiment, we concluded that higher levels of caffeine lowered the survival rate and highered the number of birth defects as well. We observed that curved spines became a common occurrence in the columns exposed to higher levels of caffeine - columns 3 and 4. Also, by day 4, 50%-75% of the zebrafish in columns A, B, and C were alive, compared to the 3% alive in column D. This is because columns A-C were exposed to lower concentrations of caffeine, and column D had the highest exposure to caffeine.

However, in the lab we had several sources of error. When putting the eggs into each well on day 1, we were a little rushed and put too many eggs in some wells. This caused our results to become faulty when looking at the exact number of eggs hatched or alive. Therefore, we had to convert all of our data into percents in order to better analyze our trends. Also, when removing dead eggs and remaining liquids from each well, we had to make sure we did not cross contaminate. However, I'm sure we unintentionally made that mistake at least once during this

lab by removing liquids in well B before well A. One abnormal piece of information we had was in well A1. By day 4, there were no alive or hatched eggs! Most likely, something went wrong while adding the liquids on day 3. This was unusual because column A was our control group and there should not have been such drastic drop in number of zebrafish alive.

We presume that the effects of caffeine for both humans and zebrafish would be very similar because the development processes between the two are very similar. Therefore an expecting mother should limit her caffeine intake as much as possible during pregnancy. This would reduce the chance of birth defects and fatality.

Further Questions

- Knowing that caffeine is found in many drinks and affects the development of humans, then how will sugar, found in numerous drinks, affect development?
- Most pregnant women drink tea because it provides an additional source of nutrients, but what are the effects of drinking too much non herbal tea or herbal teas?

Works Cited

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