Effects of Caffeine on Zebrafish Development

By: Mae Scheuing, Caroline Denton, and Megan Nchekwaram

Divine Savior Holy Angels High School

Abstract

This study was conducted to determine the effects of caffeine on zebrafish development, and to use the findings to determine the effects of caffeine in human pregnancies. Prior to conducting the study, background research was done which provided that caffeine can cause low birthweight, birth delay, and miscarriage in human pregnancies. To conduct the experiment, Zebrafish embryos were subjected to numerous concentrations of caffeine, specifically 0.05 mg/mL, 0.25 mg/mL, and 1.0 mg/mL. Their development was observed over a 96 hour period after the eggs were fertilized. The data and results established that zebrafish embryo development can be severely altered by caffeine. The presence of caffeine in a zebrafish embryo environment can be harmful to growth and development in medium concentrations (0.25 mg/mL), and potentially detrimental in high concentrations (1.0 mg/mL). The increase in caffeine concentration was deemed harmful and eventually fatal to the developing embryos, so we concluded that caffeine can affect human pregnancies by causing growth problems for babies with moderate intake, and causing more severe issues with heavy intake.

Purpose Statement

What is the effect of caffeine on zebrafish development?

Background Research

In society today, caffeine is one of the most common stimulants consumed by the general population (Morgan, Sara.). In fact, in a study done by Science Direct, it was found that approximately 85% of the US population consumes at least one caffeinated beverage every day. This survey also found that the mean caffeine intake in the US is about 165 mg per day (Mitchell, Diane). However, the mean intake also differs for different populations of people. For women from ages 19-30, 110 mg per day is the average intake. For men from ages 51-70, 260 mg per day is the average. For kids and adolescents, the average intake can range from 5-80 mg per day (McKay, Carol). Furthermore, a research study done on college students showed that more female students consume caffeine than male students. 85% of female college students responded to the survey saying they consume caffeine every day, while only 71% of male students did the same (Dillon, Pamela).

Caffeine is found in countless foods and drinks, like coffee, tea, soft drinks, energy drinks, and chocolate products. It is also becoming increasingly popular as an ingredient in prescription and over-the-counter medications for colds, influenza, weight loss, headaches, menstrual symptoms, and central nervous system stimulation medications (Morgan, Sara). The most common source of caffeine for adults is coffee, and the most common sources of caffeine for children are soft drinks and some water based beverages (Mitchell, Diane). The most common source of caffeine for pregnant women, in specific, is coffee ("Caffeine Consumption During Pregnancy and Fetal Growth").

In humans, consuming caffeine while pregnant can cause low birthweight and growth delay in babies. This is because caffeine increases the intracellular cyclic adenosine monophosphate which directly interferes with fetal development ("Caffeine Consumption During Pregnancy and Fetal Growth"). According to a study completed by researchers at the National Institutes of Health, caffeine is believed to cause blood vessels in the placenta and uterus to constrict, which could affect the blood supply to the infant. Furthermore, researchers concluded that caffeine can affect the stress hormones in infants, and cause them to rapidly gain weight after birth. This could lead to obesity and a greater risk of heart disease or diabetes later in life. In several studies, professionals have discovered that reducing caffeine intake during the last 6 weeks of pregnancy can decrease the chances of the child being born with low birthweight. Clinicians typically recommend that women reduce their caffeine intake to below 300 mg daily as early as possible in the pregnancy to prevent harmful effects for the baby ("Caffeine Consumption During Pregnancy and Fetal Growth"). In addition to a low birthweight and growth delays in babies, a high consumption of caffeine by pregnant women could also lead to miscarriages. ("Caffeine During Pregnancy").

Null Hypothesis

An increase in caffeine will have no effect on zebrafish development.

Alternative Hypothesis

An increase in caffeine will hinder the development of zebrafish .

Methods

Day One:

1. Add 4 Zebrafish embryos to each well.
2. Add 1mL of instant ocean solution to the column of wells labeled "A". This is the control group.
3. Add 1mL of 0.05 mg/mL caffeine solution to the column of wells labeled "B".
4. Add 1mL of 0.25 mg/mL caffeine solution to the column of wells labeled "C".
5. Add 1mL of 1.0 mg/mL caffeine solution to the column of wells labeled "D".
6. Dip pipette into methylene blue and then into one of the 16 wells, starting with the control group. Repeat until all 16 wells are completed.
7. Put the well plate in the incubator at 28.5^{o}C overnight.

Days Two Through Four:

1. Take the well plate out of the incubator.

2. Position a well of the control group underneath the dissecting microscope.
3. Observe how many of the embryos are alive and how many are hatched. Then record in a data table.
    a. On day four also observe physical development of the Zebrafish and record observations in a data table.
4. Starting with the control group, remove dead embryos from the wells using a pipette.
5. Tilt the well plate until the embryos settle to one side and remove the environmental factor solution from the top of the well using a pipette.
6. Repeat steps 2-6 from day one.
7. Put the well plate in the incubator at 28.5ºC overnight.

Materials

- 20 mL instant ocean solution
- 97 mL of .25 mg/mL concentration of caffeine
- 60 mL of 1.0 mg/mL concentration of caffeine
- 100 mL of .05 mg/mL concentration of caffeine
- 64 healthy zebrafish eggs
- Dissection microscope
- Dish of methylene blue
- 1 mL pipette
- 2 non labeled pipettes
- Container of wells (4x4)

Number of Hatched and Living Fish Post-Fertilization

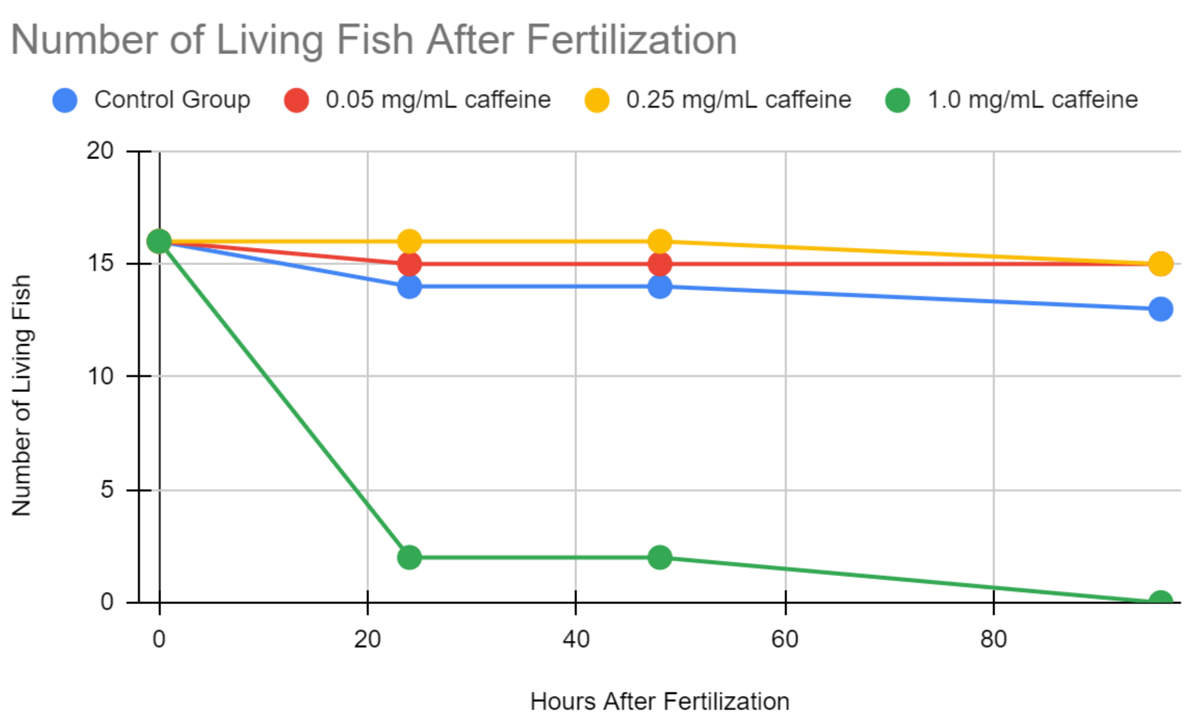
Treatment	Well #	# of starting fish	24 hours post fertilization		48 hours post fertilization		96 hours post fertilization	
			# hatched	# alive	# hatched	# alive	# hatched	# alive
Control instant ocean	A1	4	0	4	0	4	4	4
	B1	4	0	3	0	3	2	3
	C1	4	0	4	0	4	4	3
	D1	4	0	3	0	3	3	3
Caffeine 0.05 mg/mL	A2	4	0	4	0	4	4	4
	B2	4	0	3	0	3	3	3
	C2	4	0	4	0	4	4	4
	D2	4	0	4	0	4	4	4
Caffeine 0.25 mg/mL	A3	4	0	4	1	4	4	4
	B3	4	0	4	2	4	4	4
	C3	4	0	4	0	4	4	4
	D3	4	0	4	2	4	4	3
Caffeine 1.0 mg/mL	A4	4	0	1	0	1	0	0
	B4	4	0	1	0	1	0	0
	C4	4	0	0	0	0	0	0

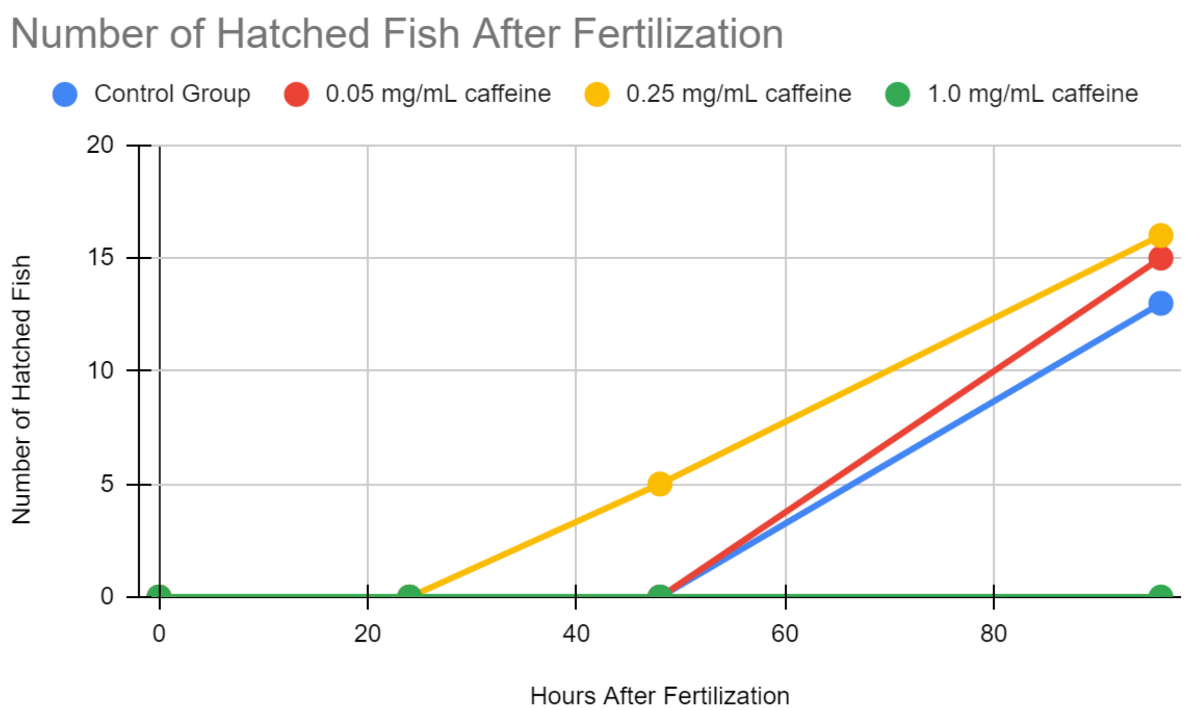
	D4	4	0	0	0	0	0	0

Qualitative Observations

Control	All the fish look healthy. They are relatively large with straight spines. All of them swim in a straight line. They all have somites.
0.05 mg/mL caffeine	Most of the fish look healthy, with one exception that does not contain somites and has a curved spine. They all swim in a straight line except the one that does not contain somites, which swims in circles.
0.25 mg/mL caffeine	The fish look unhealthy for the most part. Most of them have curved spines, a few do not contain somites, and most of them swim in circles, twitch uncontrollably, or collide with the edge of the wells. They are somewhat smaller than the fish in the control group.
1.0 mg/mL caffeine	None of the fish hatched and all of them were dead by the end of the 96 hour period. They did not develop at all, and most of the fertilized eggs died within the first 24 hour period.

Graphs





Statistical Significance for Survival

Concentration	Mean	Standard Deviation	T-Test (p-value)	Statistical Significance
Instant ocean	3.25	0.50	N/A	N/A
0.05mg/mL caffeine	3.75	0.50	0.2070	Not significant
0.25mg/mL caffeine	3.75	0.50	0.2070	Not significant
1.0mg/mL caffeine	0.00	0.00	0.0001	Extremely significant

Results

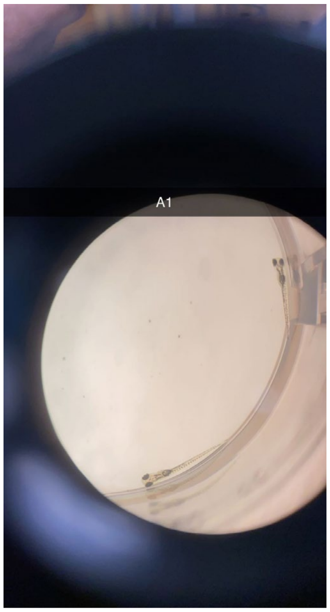


Figure 1
Control Group
Fish look healthy and swim in a straight line

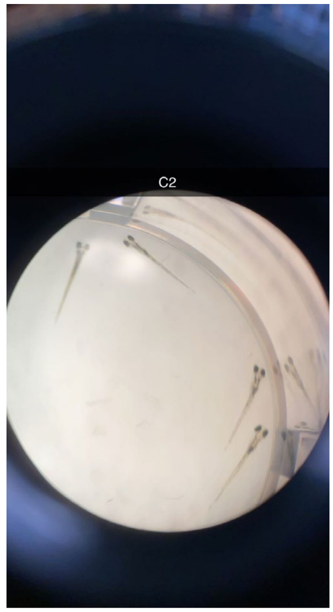


Figure 2
0.05 mg/mL solution
Fish look healthy and swim in a straight line

Figure 3
0.25 mg/mL solution
Unhealthy for the most part; most have curved spines and swim in circles or twitch

The survival rate for the control, 0.05mg/mL caffeine, and 0.25mg/mL caffeine groups were very close and were all successful. After the 96 hours post fertilization, these groups all had about 15 living fish in total. The outlier was the 1.0mg/mL caffeine group. All of these fish were dead 96 hours post fertilization. The fish hatched first in the 0.25mg/mL caffeine solution and continued hatching at the highest rate in that group until all 16 fish had hatched. However, as can be seen in Figure 3, the fish looked unhealthy for the most part. Most of them have curved spines, a few do not contain somites, and mostly all of them swim in circles or collide with the edge of the wells (Figure 3). The 0.05mg/mL caffeine solution had the second most-hatched fish with 15 of them hatching after 96 hours. All the fish looked healthy, as pictured in Figure 2, except for one that did not contain somites and had a curved spine (not pictured in Figure 2). They all swam in a straight line except the one that did not contain somites, which swam in circles (Figure 2). Thirteen fish hatched in the control group, and they all looked healthy, as can be seen from Figure 1. They were relatively big with straight spines. All of them swam in a straight line, and they all had somites (Figure 1). Because none of the fish in the 1.0mg/mLcaffeine group survived, none of them hatched. They showed no physical development, and most of the fertilized eggs died within the first 24-hour window.

Discussion

Caffeine does not affect the survival of zebrafish in low to medium concentrations (0.05mg/mLand 0.25 mg/mL), but it causes mass death of zebrafish in high concentrations (1.0 mg/mL). The data states that 15 fish survived in the 0.05 and 0.25 mg/mL concentrations, while only 13 fish survived in the control group. Therefore, in these two groups, our data is not statistically significant with a p-value of 0.2070. Therefore, in low and medium concentrations, caffeine is not a significant factor in the survival of zebrafish. However, the data states that 0 fish survived in the 1.0 mg/mL concentration while 13 fish survived in the control group. This data is extremely statistically significant with a p-value of 0.0001. Therefore, caffeine in high concentrations is a very significant factor in the survival of zebrafish.

Caffeine does not affect the hatching of zebrafish in low concentrations (0.05 mg/mL), it accelerates the hatching process in medium concentrations (0.25 mg/mL), and it completely halts the hatching process in high concentrations (1.0 mg/mL). The data shows that zebrafish in the 0.05 mg/mL concentration hatched largely around 72-96 hours after fertilization, at about the same time as the control group. Therefore, the caffeine did not affect the rate of hatching for zebrafish in low concentrations. In the 0.25 mg/mL concentration, the zebrafish began to hatch much sooner, with 5 fish already hatched at about 48 hours after fertilization. These fish hatched before the fish in the control group, which demonstrates that caffeine accelerates the rate of hatching in zebrafish when present in medium concentrations. Zebrafish in the 1.0 mg/mL concentration never hatched, even if their eggs did survive until about 48-76 hours after fertilization. The embryos in a high concentration of caffeine also did not exhibit any type of development within their eggs throughout the hours in which they remained alive. Therefore, a high concentration of caffeine makes the hatching process extremely slow or kills the embryo completely.

Caffeine causes no abnormal physical characteristics in low concentrations (0.05 mg/mL), but begins to cause abnormal characteristics in medium concentrations (0.25 mg/mL). The data shows that zebrafish in the 0.05 mg/mL concentration exhibit straight spines and contain somites for the most part. Only 1 of 15 surviving fish had a curved spine and did not contain somites. The healthy fish all swam in a straight line and were a normal size. The healthy fish in the 0.05 mg/mL concentration have characteristics that mirror the control group. In a medium concentration of caffeine (0.25 mg/mL) the zebrafish began to exhibit abnormal physical characteristics. Most of the fish in this concentration have curved spines and some lack somites. They are relatively small compared to the control group and their movement is largely restricted to swimming in circles or twitching uncontrollably.

In correlation to human health, specifically pregnant women, it is recommended that pregnant women lower their caffeine intake because a high caffeine intake could lead to miscarriages. This is shown in the higher survival rates of the zebrafish exposed to .05 and .25 mg/mL concentrations of caffeine as they were able to tolerate the conditions and survive. As stated in the background research, a pregnant woman is allowed to have no more than 300mg of caffeine which is still a significantly high amount of caffeine. For example, a 12 fl oz (approx. 355mL) Celsius energy drink has a total of 200mg of caffeine, which has an approximate concentration of 0.5 mg/mL. Meaning that at least 1-2 Celsius energy drink a day throughout a pregnancy could cause a potential miscarriage. In reference to the Celsius energy drink, even half a can of Celsius, which has an approximate concentration of .25 mg/mL, could still lead to birth defects. As the evidence showed that the zebrafish in .25mg/mL concentration had physical limitations and abnormalities.

There were two scenarios that our study could not address due to the methods of the experiment and the time limitations due to our class schedule. Between day 3 and 4 the zebrafish were exposed to the same solution for more that 48 hours instead of the original 24 hour period because we did not have access to the experiment; this could have hindered the survival rates. One thing this research does is give data for daily exposure to caffeine which we know does affect survival rates, but in reality if a pregnant woman were to drink a Celsius energy drink once a week or once in a while, the effects would be different. Thus, another question that could be asked is: if the Zebrafishes were exposed to caffeine concentrations inconsistently would that have an effect on the survival rates, and overall development? In conclusion, this study demonstrates that it would be safer for pregnant women to avoid daily caffeine intake as the outcome of consumption is indefinite and varies per individual.

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