Caffeine and Zebrafish- Do they mix well?

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Abstract

The purpose of this experiment was to figure out the effects of caffeine on Zebrafish embryos. This is important because we can figure out if it is unhealthy for pregnant women to consume large amounts of caffeine. We used zebrafish for this experiment because their eggs are fertilized and developed outside of the mother's body. They are also invertebrates just like us humans. In this experiment we tested the effects of caffeine on zebrafish. We tried to see what caffeine does to the embryos and how it affects them. We used pipettes to get the embryos and transfer solution in and out of each well. We got some very interesting data and for each of the concentrations we had very different results. The control group was very close to hatching but they ended up staying unhatched. The highest concentration(1.0 mg/mL) had very slow development and a deformed heart sac. These are the biggest data points we got throughout the experiment. Many similar experiments have been done and our results were very similar compared those. We further proved the negative effects of caffeine and more experiments to prove this will be done in the future.

Introduction

Caffeine is a central nervous system stimulant of the methylxanthine class. It is technically considered a drug and it is the world's most widely consumed psychoactive drug. Unlike most psychoactive substances, it is completely legal and in nearly all parts of the world it is just a normal thing included in foods and drinks. Caffeine is included in many different foods that humans consume on a daily basis.

We know that caffeine can increase alertness and wakefulness, at least for a short time. That is really the only reason that you'd even want caffeine because it can "wake you up" for awhile. You may them feel some negative effects based on how much you consumed. It may lead to dehydration, headaches, anxiety, and depending on how much you had, you can overdose on caffeine. For pregnant women having high amounts of caffeine can start to have more negative effects. Caffeine can contribute or lead towards a miscarriage and can also be a factor in low birth weight. These can obviously be very dangerous effects and should be definitely avoided. Though currently we aren't 100% in caffeine's effects. There is many different experiments that have been done in the past but there isn't any conclusive evidence leaning towards either side. But it not like we don't know anything. The effects stated beforehand can happen and you should be careful with your caffeine consumption levels.

Zebrafish are commonly used for experiments because their eggs are fertilized and developed outside of the mother's body. This can be very useful for research because we can very closely study the process and any effects that the surrounding area has. At first glance this may seem completely pointless and you may be thinking "Why do we care so much about these random fish." The reason is because they are invertebrates just like humans. This means that they the same major organs and tissue as us humans. According to "yourgenome.org" Zebrafish have a similar genetic structure to humans. They share 70% of genes with humans. Also, 84% of genes known to be associated with human disease have a zebrafish counterpart. This makes them very useful as we can very easily test things on them to try and cure or at least work towards helping people with certain diseases.

The purpose of these experiments is to determine how much of an effect caffeine can have on an embryo. We also want to see how much caffeine it takes to have an effect so we are using different size doses of caffeine for each embryo that we have. This will hopefully make the results more accurate and will show the starting point for effects.

I predict that the more caffeine the zebrafish have, that their overall activity will increase. They may also grow faster or more likely be prematurely hatched. I think that the heart rate of the zebrafish with also increase alongside caffeine amounts. With the largest doses it may lead to something like a heart attack or heart problems for the zebrafish (possibly a deformed heart). This should be able to produce results adequate for showing the effects of caffeine.

Materials and Methods

We used many different materials during the course of this experiment. We used each of these items; beaker for the dead embryos, plate with wells, sharpie, depression slide with cover slip, an instant ocean/embryo media solution, stock solutions of caffeine (0.05, 0.25, 1.0 mg/mL Caffeine), 28.5 C incubator, dissecting and compound microscope, disposable pipette 1mL, and a disposable pipette,minimum bore, 1.5 mm.

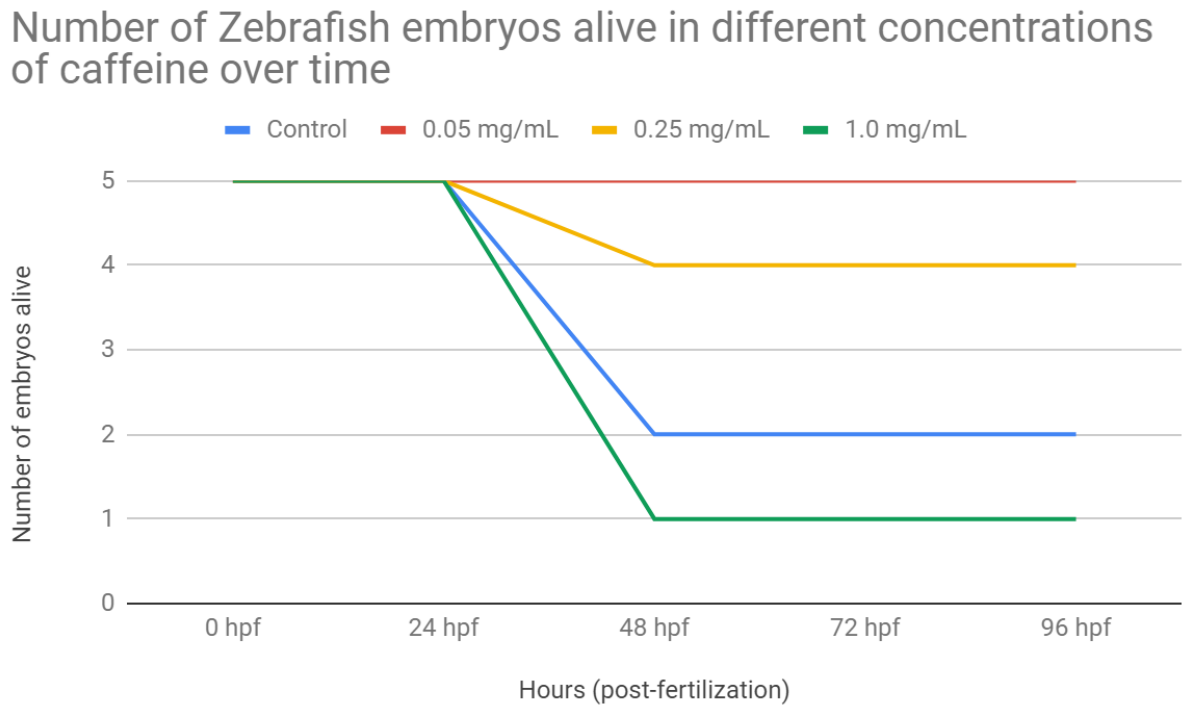
We had many procedures that we had to carefully follow throughout the experiment. First off, we labeled our plates with the correct solution. Next, we distributed the embryos into the wells, 5 per well, by using the 1.5 mm pipette to collect them and then distribute them. Then we had to use the 1 mL pipette to distribute the correct solution into each well as marked. However, before switching solutions we had to clean the pipette or get clean one. For the next step we recorded our base data onto our tables (the amount alive & the amount hatched). We then used the dissecting microscope to record observations about the embryos. Finally we put the plate into the incubator to sit overnight. The only other new step we had to do was on all the other days where we had to replace the solution. Besides that though we just recorded observations on on of the other days.

Results

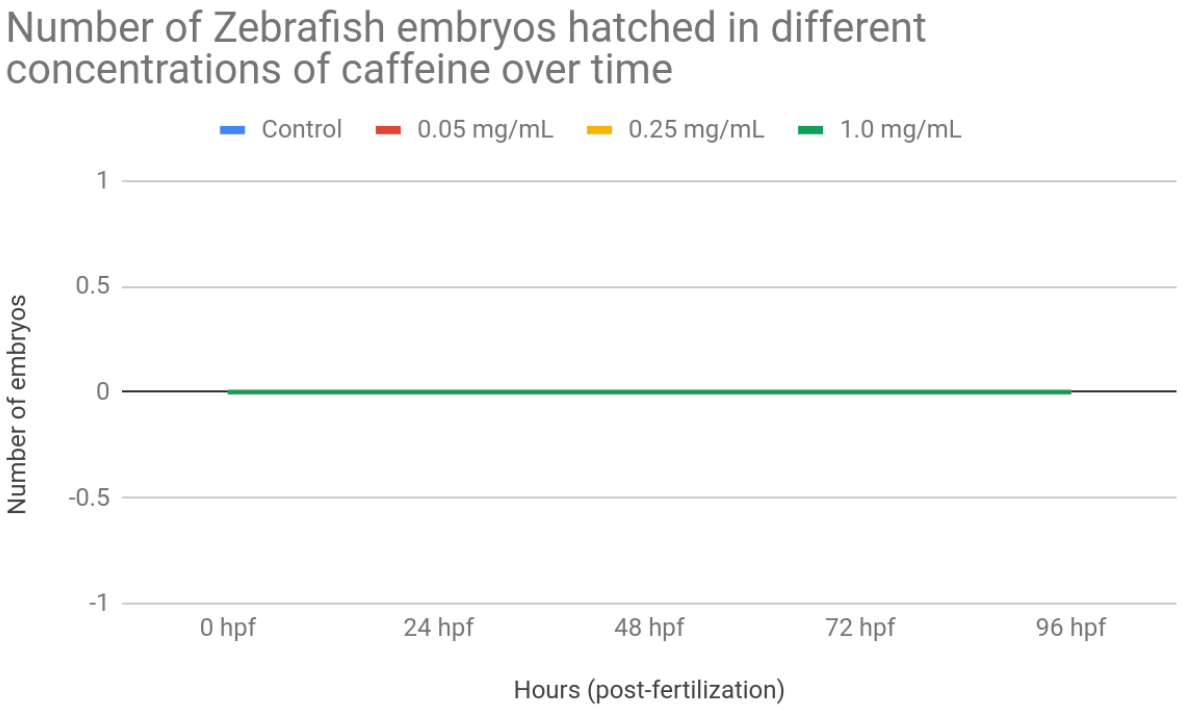
We're doing this experiment to determine how much of an effect caffeine can have on an embryo. Our research showed us that caffeine can slow development and our experiment proved that. The control group surprised us because we thought they had the highest chances of survival. 48 hpf three died on leaving only two survivors for the remainder of the experiment. The 0.05 mg/mL group all survived throughout the entire experiment. The 0.25 mg/mL group only lost one 48 hpf. The 1.0 mg/mL group lost four 48 hpf. They were also definitely the most affected by this experiment. None of the embryos hatched in any of the wells throughout the 96 hours.

	0 hpf	24 hpf	48 hpf	72 hpf	96 hpf
Control	5	5	2	2	2
0.05 mg/mL	5	5	5	5	5
0.25 mg/mL	5	5	4	4	4

1.0 mg/mL	5	5	1	1	1



	0 hpf	24 hpf	48 hpf	72 hpf	96 hpf
Control	0	0	0	0	0
0.05 mg/mL	0	0	0	0	0
0.25 mg/mL	0	0	0	0	0
1.0 mg/mL	0	0	0	0	0



Discussion

In the control group there was mostly normal development. 24 hpf there was no issues with the embryos (figure 1). Three of them ended up dying 48 hpf alongside the other wells. We were intrigued that they almost had the most casualties of all the wells considering the fact that they were in a mostly normal environment. 96 hpf there were no birth defects and they were very close to hatching (figure 2).

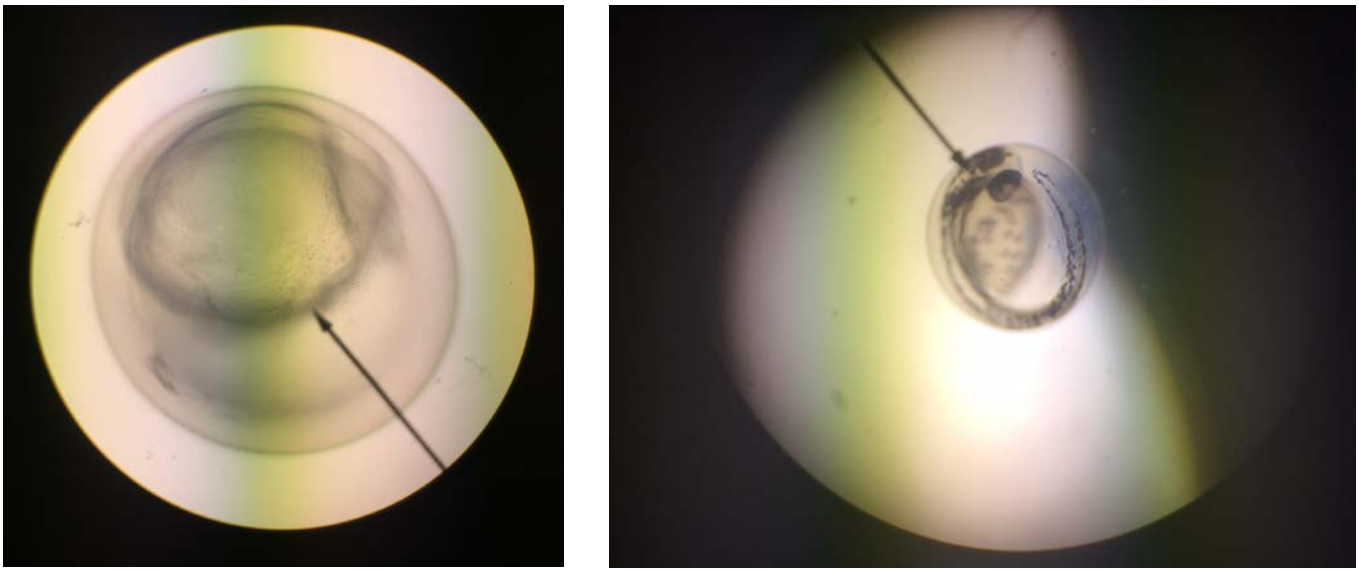


Figure 1 (24 hpf)

Figure 2 (96 hpf)

In the 0.05 mg/mL group there was slightly slower development than the control group but they were basically the same. All of the embryos survived in this group but once again none of them hatched. In the 0.25 mg/mL group there was also not lots of change in development. All but one of the embryos survived throughout the experiment. Only one died, on day three. None of them hatched.

In the 1.0 mg/mL group there was major differences in development, especially compared to the control group. First off there was only one survivor, all the others died on day three, and his development was very stunted. It also had a deformed heart sac (figure 3).

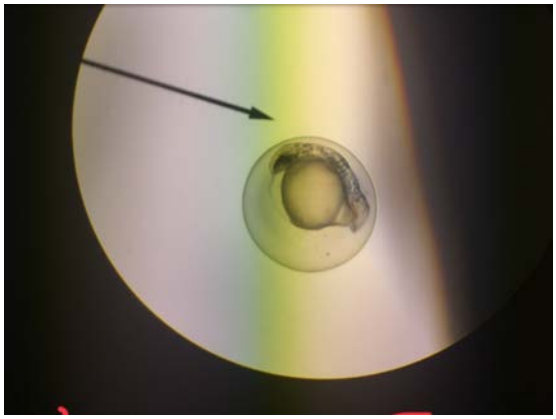


Figure 3 (96 hpf)

The experiment was not perfect however. On day two all of our zebrafish died and had to be replaced. This could cause some errors in our data compared to other experiments but the data is still useful. Our results were mostly what we expected from our research. We were surprised that the caffeine slowed development though. Based on the effects it has with humans we thought that it may lead to an early birth and the zebrafish would be developed faster. It actually had the opposite effect though. We did predict that the heart would be affected and that it may be deformed. Our results showed that caffeine slows the development of embryos and that it can have negative effects on the growth of the heart. These results matter because it is one more account proving the negative effects caffeine can have on embryos. This leads into the effects of caffeine on pregnant women because they can be affected by this.

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