Caffeine Effects on Zebrafish Embryos

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March 10th, 2018

Abstract

Research is done on zebrafish to see how caffeine affects the human fetus. According to WebMD, “It would be reasonable to advise pregnant women to drink no more than three cups of coffee a day” (2006). If one can understand how caffeine intake effects the fetus and why this is important, woman will be able to take their caffeine limits more seriously during pregnancy, which will overall provide wellbeing to the child. The course of the following experiment took place over 5 days. Different concentrations of caffeine solutions were put in three wells and one well was set aside as the control. 5 embryos are in each well, and the data is observed and recorded. The final results were unexpected, but reliable conclusions were still able to be drawn. The embryos in the higher amounts of concentration all died, but some in the lower concentrations still hatched. With these results, one can have a better understanding of what caffeine can do to a human body and how it affects developing human embryos.

Introduction

Roughly 90% of Americans consume caffeine a day (Mitchell, 2013). That is about 293,130,000 people in the United States. Caffeine, one of the oldest forms of stimuli, is an organic substance found primarily in coffee beans and tea leaves. It can now be found in many everyday products including sodas, medicine, energy drinks, coffee, candy, and yogurts. People usually consume caffeine through coffee. Caffeine is mostly used to improve mental alertness and to cure headaches. Some effects of caffeine on the body are anxiety, headaches, nausea, and restlessness (Mitchell, 2013). When women who are pregnant consume caffeine, it will affect their unborn child. It has been said that caffeine is able to enter the fetus by crossing the placenta and umbilical cord into the amniotic fluid and the baby’s bloodstream (Murray, 2015). According to American Pregnancy Association, any amount of this caffeine can cause changes to a baby’s sleeping pattern (2018). Numerous studies have also shown caffeine can cause birth defects, premature labor, preterm delivery, and reduced fertility. Exposure to high amounts of caffeine in the uterus was also associated with significantly higher risks of being overweight at 3-5 years of age. To keep these issues from happening, the recommendation for pregnant woman is not to exceed 200 mg of caffeine a day (Lieber, 2015). Research can and already is being done to show the effects of caffeine on zebrafish embryos. The results of an experiment on the zebrafish embryos reflect the effects of caffeine on a human embryos, considering they’re genes and physical structures are human like. To validate this research an experiment will be done, for which it is predicted that the zebrafish embryos exposed to the highest concentration of caffeine will have a low birth rate and a low survival rate.

Materials and Methods

Materials

Quantity	Item
3 bottles	Stock solutions of caffeine (0.05 mg/mL, 0.25 mg/mL, 1.00 mg/mL)
1	Beaker for dead embryos & liquid disposal
1	Sharpie
1 bottle	Instant Ocean/Embryo Media Solution
2-3	Transfer pipette
1	Multi-well plate
20	Zebrafish embryos
1	Dissecting microscope
1	Camera
1	28.5*C incubator
1	Glass slide
1	Compound light microscope

Methods

Day 1

On day 1, obtain the rinsed zebrafish embryos. Label the well plate with a piece of masking tape. The tape should include the concentration of your experimental and controls wells. Fill the well on the upper left side with 1 mL of Instant Ocean solution using a wide pipette. Fill the experimental wells (the three to the right of the control well) with 1 mL of the caffeine solutions. Make sure to put the correct solutions I each well. Using the narrow pipette, put 5 embryos in each well. Record the amount of alive embryos on the data table. Observe the embryos through the microscope and record any observations. Finally the place the well plate in the 28.5*C incubator and leave overnight.

Day 2, 3, and 4

On day 2, day 3, and day 4, remove the well plate from the incubator. Remove all the dead embryos with the narrow pipette and put them in the waste cup. Count the remaining embryos and any hatched fish and record on the data table. Remove the solutions from each of the wells and replace with a fresh solution using a clean pipette for each of the solutions. Once solutions have been changed and dead embryos removed, place the well plate under the dissecting microscope and record observations. To observe a single embryo with detail, use a skinny pipet to move an embryo to a glass slide and observe the slide under a light microscope. When finished, return the well plate to the incubator.

Day 5

On day 5, remove the well plate from the incubator. Remove all the dead embryos with the narrow pipette and put them in the waste cup. Count the remaining embryos and any hatched fish and record on the data table. Remove the solutions from each of the wells and replace with a fresh solution using a clean pipette. Use a clean pipette for each of the solutions. Once solutions have been changed and dead embryos removed, place the plate under the dissecting microscope and record observations. To observe a single embryo with my detail, use a skinny pipet to move an embryo to a glass slide and observe the slide under a light microscope. When finished, return the well plate to the incubator. Record all observations. When finished, place all the embryos and fish in the waste cup and dispose of properly.

Results

Research was done to see how caffeine really affects the human fetus; to do so, zebrafish embryos were tested because of how similar they are to human embryos. The original hypothesis stated that exposed to caffeine, the embryos would have a lower birth rate and survival rate. The independent variable in this experiment was the concentration of the caffeine in each well because it does not depend on any other variable. The dependent variable was the number of embryos hatched and alive because it depends on the concentration of caffeine and its environment. The control was the Instant Ocean solution as it has 0.00mg/mL of caffeine. All of the embryos in the control group died by day 5 and none of them hatched. The dramatic drop of alive embryos in the control group is shown in figure 2. As for the well with 0.05 mg/mL of caffeine, only 2 died, and this happened slowly over the period of the 5 days (fig. 2). Only two of the embryos hatched in the 0.05 solution which happened near the end of the experiment (fig. 4). As for the 0.25 mg/mL caffeine solution, 3 fish were dead by the end, which happened very gradually. About 1 died a day (fig. 2). By day 5 in this solution, 2 had hatched, but it happened overnight between day 4 and 5 at the end of the experiment when the hpf was 96 (fig. 4). Finally, the group that had the most caffeine (1.00 mg/mL) had zero hatched eggs (fig. 4), and they all died with in the course of only 3 days (fig.2).

Figure 1:

Number of Embryos Alive vs Dead (Alive/Dead) at Different Concentrations of Caffeine over One Week

Time	hpf	Control Group (well #1) Alive / Dead	Experimental Group Concentration 0.05 mg/mL (well #2) Alive / Dead	Experimental Group Concentration 0.25 mg/mL (well #3) Alive / Dead	Experimental Group Concentration 1.00 mg/mL (well #4) Alive / Dead
Day 1	0 hpf	5 / 0	5 / 0	5 / 0	5 / 0
Day 2	24 hpf	5 / 0	4 / 1	4 / 1	2 / 3
Day 3	48 hpf	1 / 4	4 / 1	3 / 2	1 / 4
Day 4	72 hpf	1 / 4	4 / 1	2 / 3	0 / 5
Day 5	96 hpf	0 / 5	3 / 2	2 / 3	0 / 5

Figure 2:

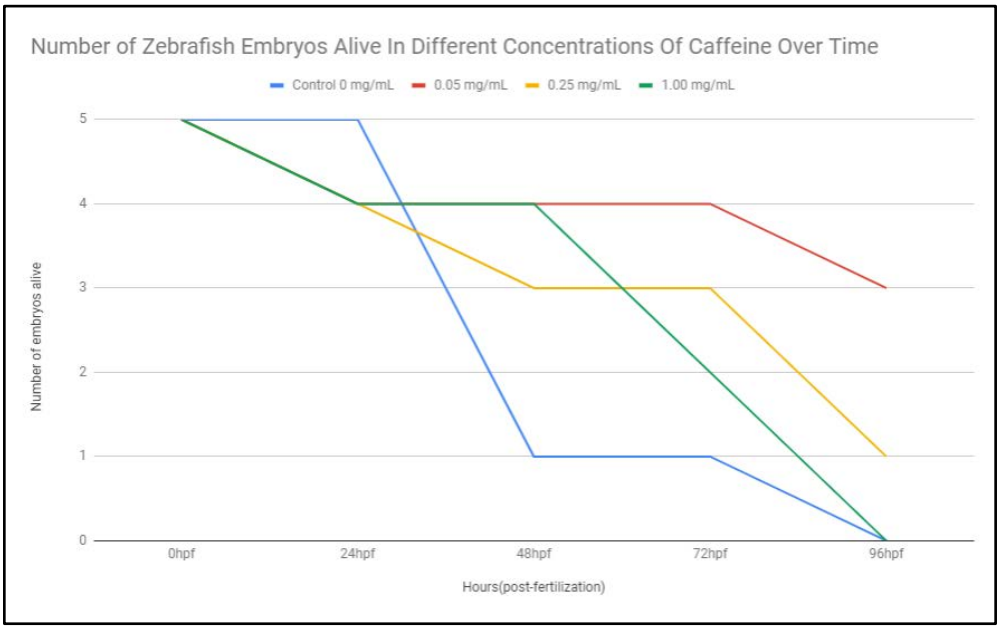

Figure 2 shows the different rates at which the embryos died. Both the embryos exposed to the 1.00 mg/mL caffeine solution and the ones in the control all died, while the other solutions had slower death rates.

Figure 3:

Number of Embryos **Hatched vs Unhatched (H / U)** at Different Concentrations of **Caffeine** over One Week

Time	hpf	Control Group (well #1) H / U	Experimental Group Concentration **0.05 mg/mL** (well #2) H / U	Experimental Group Concentration **0.25 mg/mL** (well #3) H / U	Experimental Group Concentration **1.00 mg/mL** (well #4) H / U
Day 1	**0** hpf	0 / 5	0 / 5	0 / 5	0 / 5
Day 2	**24** hpf	0 / 5	0 / 5	0 / 5	0 / 5
Day 3	**48** hpf	0 / 5	1 / 4	0 / 5	0 / 5
Day 4	**72** hpf	0 / 5	2 / 3	0 / 5	0 / 5
Day 5	**96** hpf	0 / 5	2 / 3	2 / 3	0 / 5

Figure 4:

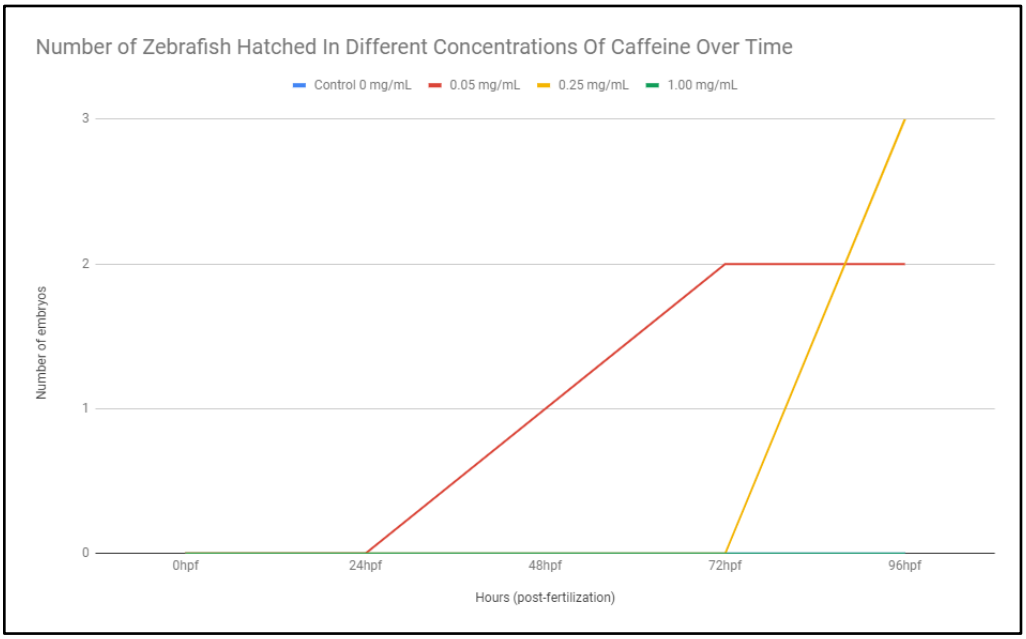

Figure 4 shows how only the two solution (0.05 mg/mL and 0.25 mg/mL) has any hatched embryos. The embryos in the 0.05 caffeine solution hatched earlier, but more embryos in the 0.25 caffeine solution hatched overall.

Figure 5:

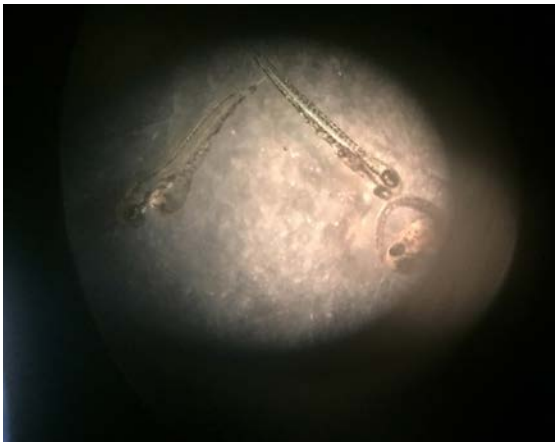

Figure 5 shows the hatched fish in the 0.05 solution on day 5. They have been exposed to caffeine for 5 days now but are still alive and 2 of them are hatched.

Figure 6:

Figure 6 shows an embryo that looks cloudy and white exposed to 1.00 mg/mL of caffeine at 24 hpf on day 2. As one can see, it's only the second day and already the embryo has died.

Figure 7:

Figure 7 shows embryos that are alive and unhatched in the control well on the first day. The zebrafish has not formed yet, but one can tell it is alive because it isn't foggy.

Discussion

The results provided from the research did not support the original hypothesis. The hypothesis was that the zebrafish embryos exposed to a higher concentration of caffeine will have a lower birth rate and a lower survival rate. But as it turned out, the embryos that did the best and survived the longest were the ones in the 0.05 mg/mL and 0.25 mg/mL of the caffeine solution. More embryos with only 0.05 mg/mL of caffeine hatched. The original hypothesis was based on the knowledge that caffeine consumed during pregnancy has effects such as a lower birth weight, birth defects, premature labor (hatching early) and other issues. The data received did line up with the hypothesis in that the largest amount of caffeine solution (1.00 mg/mL) because by day 5, none of the embryos had hatched and all of them were dead. Based on all the sources gathered, that made sense since caffeine is supposed to have a negative effect on the fetus. However, according to American Pregnancy Association, "In another study released by Epidemiology journal, there was no increased risk in women who drank a minimal amount of coffee daily (between 200-350 mg per day.)" (2018). Similar statements were said in other sources, one stated"ACOG says that so far, mild caffeine intake (less than 200 mg) isn't considered to be a major cause of miscarriage or premature birth" (Murray, 2015). This can explain the reason as to why some of the zebrafish were able to survive in smaller amounts of caffeine. This does not explain why none of the fish in the control did not live though. Based on what was learned from the research done, it can be drawn that the low survival within the control well was possibly caused by an error during the experiment. There may have been some other chemicals in the wells that those fish were in that contaminated the water. There could have also been some cross contamination when using the pipettes. The contamination was probably done on day 2, because from day 2 to day 3 the number of embryos alive dropped dramatically from 5 to 1. To improve this experiment for the future one should make sure to use a different pipette for each well, and make sure the well plate is clean. With the results provided, it is proven that while pregnant, it is safe to consume small amounts of caffeine, but large amounts will show negative effects on the fetus such as miscarriage. The experiment clearly was flawed considering all the control fish died. However, it is shown exposure to high amounts of caffeine did eventually kill all the fish, but exposure to smaller amounts of caffeine have little effect on embryos, though large amounts can lead to defects in birth and death.

References

Boyles, S. (2006, January 25). Some Coffee OK Late in Pregnancy? Retrieved February 5, 2019, from https://www.webmd.com/baby/news/20070125/some-coffee-ok-late-pregnancy#1

Murray, L., Adler, S., BabyCenter Staff, & BabyCenter Medical Advisory Board. (2015, December). Caffeine During Pregnancy. Retrieved February 5, 2019, from https://www.babycenter.com/caffeine-during-pregnancy

American Pregnancy Association. (2018, August 17). Caffeine Intake During Pregnancy. Retrieved February 6, 2019, from http://americanpregnancy.org/pregnancy-health/caffeine-intake-during-pregnancy/

Lieber, M. (2018, April 23). Caffeine in pregnancy linked to childhood weight gain. Retrieved February 6, 2019, from https://www.cnn.com/2018/04/23/health/caffeine-pregnancy-child-overweight-study/index.html

Diane C. Mitchell, Carol A. Knight, Jon Hockenberry, Robyn Teplansky, Terryl J. Hartman. (2013, November 01). Beverage caffeine intakes in the U.S. Retrieved from https://www.sciencedirect.com/science/article/pii/S0278691513007175