The Effects of Caffeine on the Number of Zebrafish Hatched

Alex Witt
Waukesha North High school

Abstract

Many people drink coffee or soft drinks which contain a lot of caffeine. The purpose of this experiment was to observe how caffeine affects zebrafish embryos, which could correlate to how caffeine affects human embryos. In the experiment, caffeine solutions of different concentrations were used to fill wells that had zebrafish embryos in them. By using different concentrations, there could be gradual results. The results showed that caffeine caused the zebrafish embryos to hatch later. Inferences can concurred that caffeine is bad when pregnant.

Introduction

Caffeine is a drug in most drinks, such as coffee and some sodas. It is called the most popular drug in the world. Caffeine temporarily makes humans more alert by blocking sleep-inducing chemicals inside our brains. How will this drug affect new-forming babies? Based on the information at epicene.org (2015), there are adverse effects. Caffeine can negatively affect the quality of oocyte or egg. It can also affect the embryo health because caffeine is an appetite suppressant preventing the embryo from getting all of the nutrients needed. In a recent experiment, the effects of caffeine on zebrafish embryos in 0mL, 0.05mL, 0.25mL, and 1mL of caffeine after 72 hours was studied. Observations were made after every 24 hours. The hypothesis that was formed was if the eggs were exposed to caffeine for a prolonged period of time, then hatching will be delayed. This experiment was done based around what would happen to embryos exposed to caffeine and how these effects relate to human embryos exposed to caffeine.

Materials and Methods

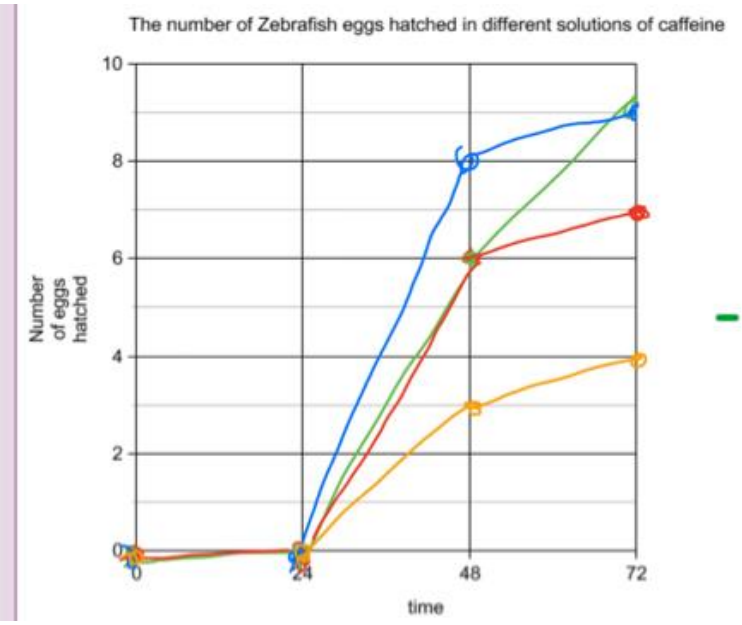
The equipment necessary to conduct this experiment was 1 bottle each of 0.05, 0.25, and 1 mL of caffeine solution. Next, the experiment required one beaker for dead embryos and liquid disposal, a Sharpie, and a bottle of Embryo Media Solution for the control embryos. A 1.5 mm pipette was needed to transfer and manipulate the embryos and measure the amount of caffeine in each well. The 1 mm pipette was needed to clean out the old solutions without getting rid of the embryos. To start off this experiment, the wells were labeled with their respective concentration of caffeine, 1-4. On the first day, 10 eggs were put into each of the wells and the wells were filled with the caffeine solution. The total amount of solution used in each well was 1 mL measured using a pipette. Next, the Zebrafish eggs were examined under a stereoscope and the observations were recorded in a data table. After examination of the eggs was completed, the caffeine solutions were drained and replaced with the same caffeine concentration (except the first day) and then put in a 28.5 C incubator for the remainder of the time. This procedure was repeated every 24 hours until the embryos were exposed for 72

hours. During this experiment, gloves were worn when handling caffeine because the solution can be absorbed through the skin. The number of eggs that were hatched was measured during this experiment.

Results

After the experiment was completed, the data was examined. In the higher concentration of caffeine, deformities and deaths occurred. This experiment was performed to see the effects of caffeine on zebrafish embryos. The hypothesis that was formed was that if the eggs developed in caffeine, then they will hatch overdue. The variables through this experiment were the zebrafish eggs that were in 0.0mL of caffeine was the control, the time to hatch for the eggs was the dependent variable, and the caffeine solutions was the independent variable. Overall, in the higher concentration, less eggs hatched. The null hypothesis for this experiment was rejected because the value of the chi-square was 9.92 with a critical value of 7.82. Figure 1 below shows the number of eggs hatched in 72 hours in each of the wells. Table 1 is a table showing hatched zebrafish eggs versus non-hatched eggs.

Figure 1: The number of eggs hatched in 72 hours in each of the wells



Concentration	Color
0.0 mg/mL of Caffeine	Red
0.05 mg/mL of Caffeine	Green
0.25 mg/mL of Caffeine	Blue
1 mg/mL of Caffeine	Orange

Table 1 Hatched zebrafish eggs versus non-hatched eggs.

Treatment	Hatched	Non-Hatched	Total for rows
0.0 mg/mL of Caffeine.	7	3	10
0.05 mg/mL of Caffeine	9	1	10
0.25 mg/mL of Caffeine	9	1	10
1 mg/mL of Caffeine	4	6	10

.

Discussion

This experiment clearly shows the negative impact caffeine has on embryos. The caffeine caused a delayed developmental process which meant less eggs were hatched in the higher concentrations. This adverse effect relates to our research, because if the caffeine delays the time it takes for zebrafish eggs to hatch, then caffeine could also delay the time it takes for a human embryo to fully develop. Questions that can be developed for further research is what would happen if a hatched zebrafish would act if the tanks containing the fish had a certain concentration of caffeine for a month as it grew. When draining the solutions out the wells, drain a bit of liquid slowly to prevent eggs from being sucked out of injured. Based on the results, the hypothesis is accepted, because the eggs did hatch later in higher concentrations of caffeine than the control. There could have been error in this experiment when replacing solutions, as the embryos could have been harmed by the pipette. In conclusion, based on the evidence caffeine does delay the time it takes for zebrafish embryos to hatch, and could have the same effect on human embryos. This coincides with our background research as studies have shown caffeine can have adverse effects on human embryos.

Bibliography

Caffeine and sleep, (n.d,), Retrieved from http://sleep foundation.org/sleep-topics/caffeine-and-sleep

Caffeine and pregnancy, (n.d,), Retrieved from www.epigee.org/pregnancy/caffeine.html

Caffeine: Does it affect your fertility and pregnancy, (n,d), Retrieved from www.resolve.org/about-fertility/optimizing-fertility/caffeine-does-it-affect-your-fertility-and-pregnancy.html