MEAN CAFFEINE

Nitin and Logan Welter

Abstract:

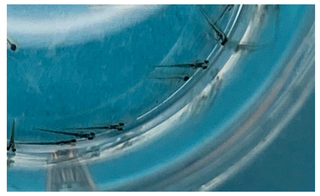
Caffeine, a psychoactive stimulant, is commonly consumed in the United States, even among pregnant women. The purpose of this experiment is to understand how different concentrations of caffeine consumption early on in pregnancy can affect a woman's developing embryo and to raise awareness regarding those potential risks. For ethical purposes, this experiment utilizes zebrafish (Danio rerio) embryos to understand how exposure to caffeine can affect embryo development. Zebrafish embryos were used due to their acceptance as model organisms in the scientific community when studying developmental biology for human comparison. The results of this experiment show the overall mortality rates for the zebrafish embryos increased as the concentration of the caffeine solution increased. Clearly, caffeine at certain levels significantly increases the risk of death for the zebrafish embryos and those results are translatable to human embryos.

Introduction:

The scientists for this experiment considered the research question: How will different concentrations of caffeine affect the zebrafish embryos? Other research has shown caffeine is a stimulant in small quantities, but in higher concentrations, it can slow down all parts of the body. The scientists hypothesized that if you expose a zebrafish embryo to increasing amounts of caffeine, then the mortality rate of a zebrafish embryo will increase as the caffeine concentration increases. Results from zebrafish studies yield important scientific knowledge regarding human biology. Zebrafish share 70% of their genes with human genes and, as fellow vertebrates, share similar developmental processes with humans. Therefore, results of this study will add to the understanding of how a commonly consumed ingredient like caffeine can affect human embryo development. The scientists conducted this experiment to test their hypothesis.

Materials and Methods:

Four different solutions were created for the experiment. The wells of the chemplate were labeled with four different numbers. The solution in well #4 was the control - simply embryo media with no caffeine, while the other three solutions had various levels of caffeine mixed into the embryo media. Well #1 held a solution of 0.05 mg caffeine/mL of embryo solution, well #2 held a solution of 0.25 mg caffeine/mL of embryo solution, and well #3 held 1 mg caffeine/mL of embryo solution. Next, zebrafish embryos two hours post fertilization were added to each well, counted, and recorded on a chart. The wells were observed at 24, 48, and 72 hours post fertilization to measure how many embryo had hatched and how many embryo were alive (both hatched and unhatched). This data was added to the chart.



Picture A: Part of the well of the control group after 48 hours.

Picture B: Part of the well of the 1 mg/mL group after 48 hours.

Results:

The variable that was changed in this experiment was the amount of caffeine inside each of four wells containing an embryo solution and zebrafish embryos. One well (the control) had no caffeine added, and the three other wells each had three different caffeine concentrations added to them. The zebrafish embryos were negatively affected by the caffeine. At 72 hours post fertilization, the percentage of zebrafish embryos that had hatched decreased as the caffeine concentration increased. At that same time interval, the percentage of zebrafish embryos alive decreased as the caffeine concentration increased (see graphs 1 and 2). The control group that wasn't exposed to caffeine had a higher percentage of embryos alive and a higher percentage of embryos hatched after 72 hours when compared with the wells that contained caffeine. The independent variable in the experiment is the concentration of caffeine in the embryo solution and the dependent variables are the number of embryos hatched and the number of embryos alive at 24, 48, and 72 hours post fertilization. These results demonstrate how different concentrations of caffeine affect zebrafish embryos, as well as how the overall mortality rate for zebrafish embryos increases as the concentration of caffeine solution increased.

Sources:

https://pubmed.ncbi.nlm.nih.gov/24189158/

https://healthblog.uofmhealth.org/childrens-health/parents-perk-up-to-dangers-of-caffeine-for-teens

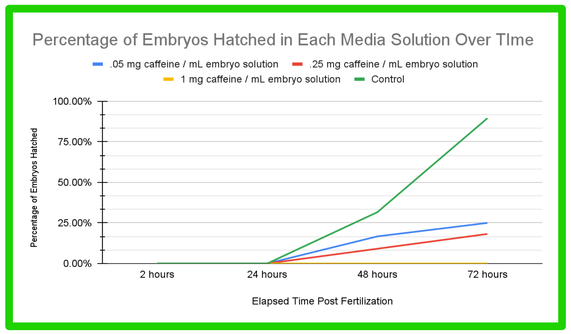
Data Analysis:

An unpaired t-test was used to compare the means of two independent groups to determine if there is a significant difference between the two. The mean used was the percentage of embryos alive after 24, 48, and 72 hours post fertilization for each of the three caffeine solutions compared with the control solution. The percentage of embryos that lived in the control solution compared to each of the three caffeinated solutions was statistically significant based on the high t-values and low p-values for each of the caffeine solutions compared to the control solution with no caffeine. (See Table 1.) The p-values are so low (close to zero) you can reject the null hypothesis and conclude the results are statistically significant.

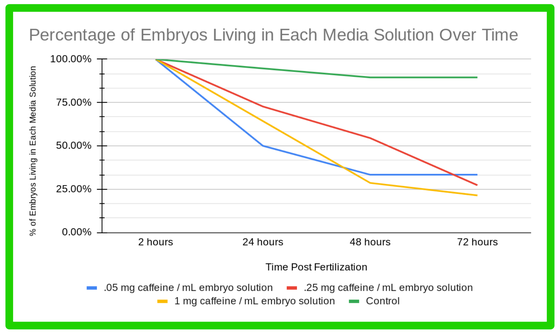
Unpaired T Test Results

Solution: (mg Caffeine / mL embryo solution)	Mean	T-score	P-value	Statistically Significant
.05mg	38.867	8.9818	0.0009	Yes
0.25mg	51.5	2.9864	0.0405	Yes
1mg	38.1	3.9721	0.0165	Yes

Table 1



Graph 1



Graph 2

Discussion:

This experiment data supports the hypothesis: if caffeine concentration exposure increases, then the mortality rate of the zebrafish embryos increases. All three of the embryo solutions containing caffeine had a negative effect on the hatching and viability of the zebrafish embryos. After 72 hours, embryos exposed to each of the three caffeine solutions had statistically significant higher mortality rates compared to the control solution with no caffeine (see table 1). In the case of the 1 mg caffeine/mL of embryo solution, none of the embryos hatched - they simply died. If the experiment was to continue for a couple more days, it is likely the last three unhatched embryos in this 1 mg caffeine/mL embryo solution would die before hatching. The overall trend shown in both graphs 1 and 2 demonstrated an inverse relationship between the concentration of caffeine and the number of embryos that hatched or lived. After 72 hours, the higher the caffeine concentration, the lower the percentage of embryos that hatched or lived. The control solution had the highest percentage of both hatched embryos and the highest percentage of embryos that lived. One limitation with this experiment is that the number of zebrafish embryos was not the same in each well at the start of the experiment. This could have caused differences in embryo development if there was a shortage or excess of nutrients in one or more of the wells. The research conducted proves that certain amounts of caffeine will increase the mortality rate of vertebrate embryos, but at what concentration level is there a statistically significant increase in mortality, and how can we estimate that threshold relative to human embryos? The negative effects of caffeine, coupled with its common usage in many parts of the world, underscore the need to further determine what can be considered a safe level for healthy embryo development in pregnant women. Furthermore, while this experiment and others similar to it have looked at the immediate negative effects of caffeine exposure to developing embryos, scientists need to study what other negative effects caffeine may have that show up later in a human's life.