Effects of Caffeine on Zebrafish Embryo Development

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Abstract

As with most substances, a fetus will absorb caffeine, just as the mother's body will (Caffeine Intake). The effects of caffeine on human development is important, as it is a substance that one's body does not naturally need. The purpose of this experiment was to determine how caffeine affected zebrafish mortality and development. By recording their development survival in different concentrations of caffeine, we concluded that caffeine negatively affects these factors. Hopefully, this research can be used to increase fetus health and lead to deeper studies on caffeine in human development.

Day	Embryos Alive (2)			
	50 ppm	250 ppm	500 ppm	Control
1	8	8	8	8
2	7	8	7	8
3	7	8	7	8
4	7	8	7	8
5	7	7	3	7

Introduction

Caffeine has been linked to developmental defects, low birth rate, and miscarriages (Caffeine Intake). As a stimulant and drug, caffeine can affect developing organisms, as it may sway the homeostasis of internal mechanisms and hormones. Since pregnant women across the world intake caffeine at varying rates, we wanted to test the effects of caffeine absorption in developing organisms. The lifespan before the fetus is born is highly subjective to substances taken in by the mother, and the effects of caffeine are scarcely known for this time in development

For this experiment, we used zebrafish as our test subjects. They have a quick developmental period- about three days- and are relatively transparent. Additionally, zebrafish have been known to show similarities to human genetics. It can be hypothesized that as caffeine concentrations increased, so too did the rates of mortality and severity of deformities.

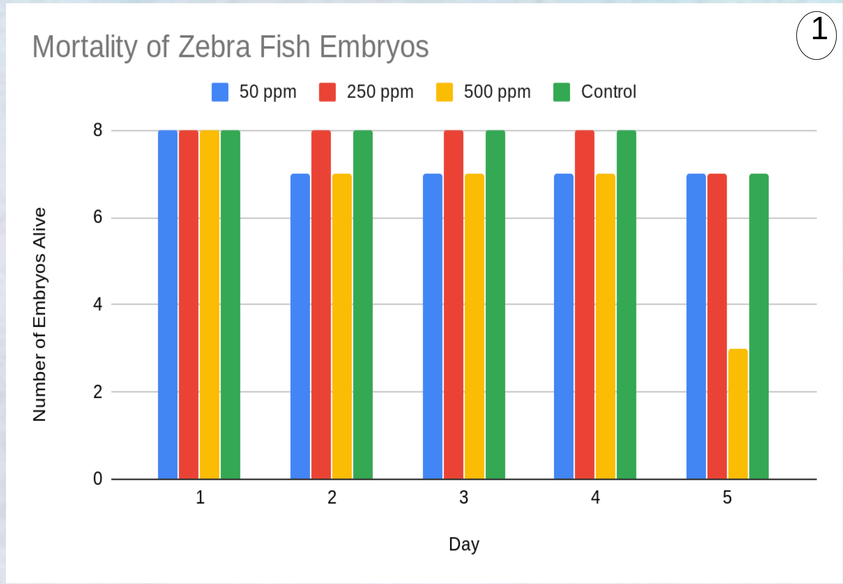
Method

Method:

Eight embryos were put into four wells on a falcon dish. Each well had a different caffeine concentration (50 ppm, 250 ppm, 500 ppm, control) which were renewed daily. We observed their development each day, and collected our data by counting the number of embryos still alive. For the data on deformities, this was done by observing the fish under a stereoscope or microscope and comparing them to the control group. Outcomes were deduced once all data was collected by graphing the data and drawing conclusions

Materials:

- Microscope
- Falcon Dish
- Pipet
- Saltwater concentration
- Caffeine concentrations
- Embryos



Legend: (1) represents the mortality of the embryos throughout the experiment in their respective concentrations. (2) represents another view of the survival rates of the embryos throughout time.

Photos (A), (B), (C), and (D), represent the development of the embryos on day 5; their respective contents are 50 ppm, 250 ppm, 500 ppm, control

References:

Abdelkader, Tamer Said, et al. "Exposure Time to Caffeine Affects Heartbeat and Cell Damage-Related Gene Expression of Zebrafish Danio Rerio Embryos at Early Developmental Stages." *Journal of Applied Toxicology*, 2012

"Caffeine Intake During Pregnancy." *American Pregnancy Association*, 23 Sept. 2019

Chen, Yau-Hung, et al. "Movement Disorder and Neuromuscular Change in Zebrafish Embryos after Exposure to Caffeine." *Neurotoxicology and Teratology*, vol. 30, no. 5, 2008, pp. 440–447., doi:10.1016/j.ntt.2008.04.003.

Results

Using falcon dishes we placed eight embryos in in each well.

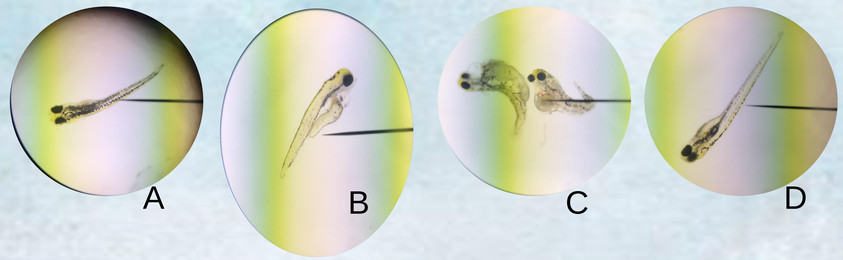
Each well had a different concentration of caffeine (50 ppm, 250 ppm, 500 ppm, 0 ppm (Control)). We observed them for five days.

Independent variable: Different concentrations in wells

Dependent: The number of embryos alive and severity of deformities

Control: The wells with saltwater concentration (0 ppm)

As the concentrations increased, mortality and severity of deformities increased. The control group had more survivorship and no deformities. This confirms our hypothesis, and concludes that caffeine affects development and mortality.



Discussion

Our data supported our hypothesis that, as caffeine increased, the mortality and severity of deformities also increased. Fewer embryos survived maturity in higher concentrations, as graph (1) shows. Additionally, their deformities (i.e. bent spine) became more severe as time and concentration increased, as seen in the pictures above. This aligns with other studies, and suggests that caffeine can induce apoptosis and misalignment of muscle fibers (Abdelkader, Chen). Our experiment was limited as we only had two weeks to observe the embryos, and could not do repeat tests to ensure substantial results. The findings of this study is important as it can lead to further research of caffeine on fetus development, and what it may do to developing organisms within the ecosystem. With the present possibility of caffeine entering ecosystems and people, these results can be used to develop more in-depth experiments of the effects of caffeine.n