

Development Effects of Vitamins on Zebrafish Embryos

By: Leah Golden, Isabella Zuehlke

Abstract

The purpose of this experiment was to test the effect of B6 and B12 vitamins on zebrafish. Vitamins are key to human health, but what if a person takes too much or too little? We studied zebrafish that were exposed to the vitamins in 4 different solutions, a control (0%), 2.5%, and a 10% solution, for three days. Through this process, we found that with a small amount of concentration there were higher survival rates than zebrafish with no concentration or a high concentration.. We used zebrafish in our experiment because they grow rapidly and we can see a change in their bodies because they are transparent. Their structures are also similar to humans. This experiment shows how too much or too little intake of vitamins can positively or negatively impact the growth and health of a human.

Introduction

Prenatal vitamins such as B6, B12, and folic acid are critical elements in embryonic growth. Subpar levels of B6, B12, and folic acid can cause extreme problems. Not enough folic acid creates a possibility of a low-weight baby as well as anemia throughout childhood and adolescence. Next, deficiency in B12 could lead to infertility or birth defects. (2). It was also shown that individuals with higher levels of B12 were more developed than those without it (1). Additionally, B6 plays an important role in the functions of a cell and in cognitive development (3). Numerous amounts of birth defects and infertility could be prevented through the use of these vitamins. It is important to understand the side effects because this simple step could save the lives of many. This experiment on Zebrafish determines the effect of these important vitamins on embryonic development by comparing the effect of a surplus, deficit, and normal amount of vitamins. It is hypothesized that zebrafish exposed to the prenatal vitamins will be the strongest and have the highest chance of survival.

Materials and Methods

We first filled 2 rows of a Falcon dish (using a disposable pipet) with 3 mL of our control liquid which is an embryo media, an instant ocean solution that has a concentration of 200 mg/L. We then created 3 different concentrations made with B12 and B6 vitamins; to create this we crushed the vitamins and dissolved them in the 200 mg/L ocean solution in a beaker. Additionally, we used a coffee filter into a funnel that was placed in a beaker and poured our solution into the funnel to remove the extra vitamins that did not dilute. With the new vitamin solution, we made a 10% concentration, a 5% concentration, and a 2.5% concentration. We created these concentrations by diluting the vitamin solution with the embryo media. We then poured the 2.5% solution into the same falcon dish as the control but in 2 different rows. We then poured the 5% and 10% solutions into a second falcon dish. We then put 5-7 Zebrafish embryos into each well of both falcon dishes. After the embryos are settled we put them in an incubator set at 28°C to help them grow faster. We observed the zebrafish for three days under a microscope to see if they hatched, died, and to see how their bodies were developing. We kept a chart with all of our data and pictures of what each zebrafish looked like in each of their solutions. If there was a dead embryo we took a pipet and carefully sucked it up without disturbing the other embryos.

Results

Figure 1 indicates how the independent variable of the different concentrations of the solutions affected the dependent variable of the number of embryos dead within our 3 days. By using a Fisher Test the P-value was 0.338 which means between control and 2.5%, there was not a significant difference between the number of living and dead. From this, it can not be concluded that higher concentrations of these vitamins will not make the embryos healthier, and live longer.

Figure 2 shows how the independent variable of the different concentrations of the solutions affected the dependent variable of the number of embryos hatched and unhatched. When using the Fisher test, the P-value was 0.0028 which means there is a significant difference between the number of embryos hatched between the control and 2.5%. This means that the exposure to vitamins impacted the number of embryos hatched. Therefore, it can be concluded that the vitamins negatively affected the growth of the embryos.

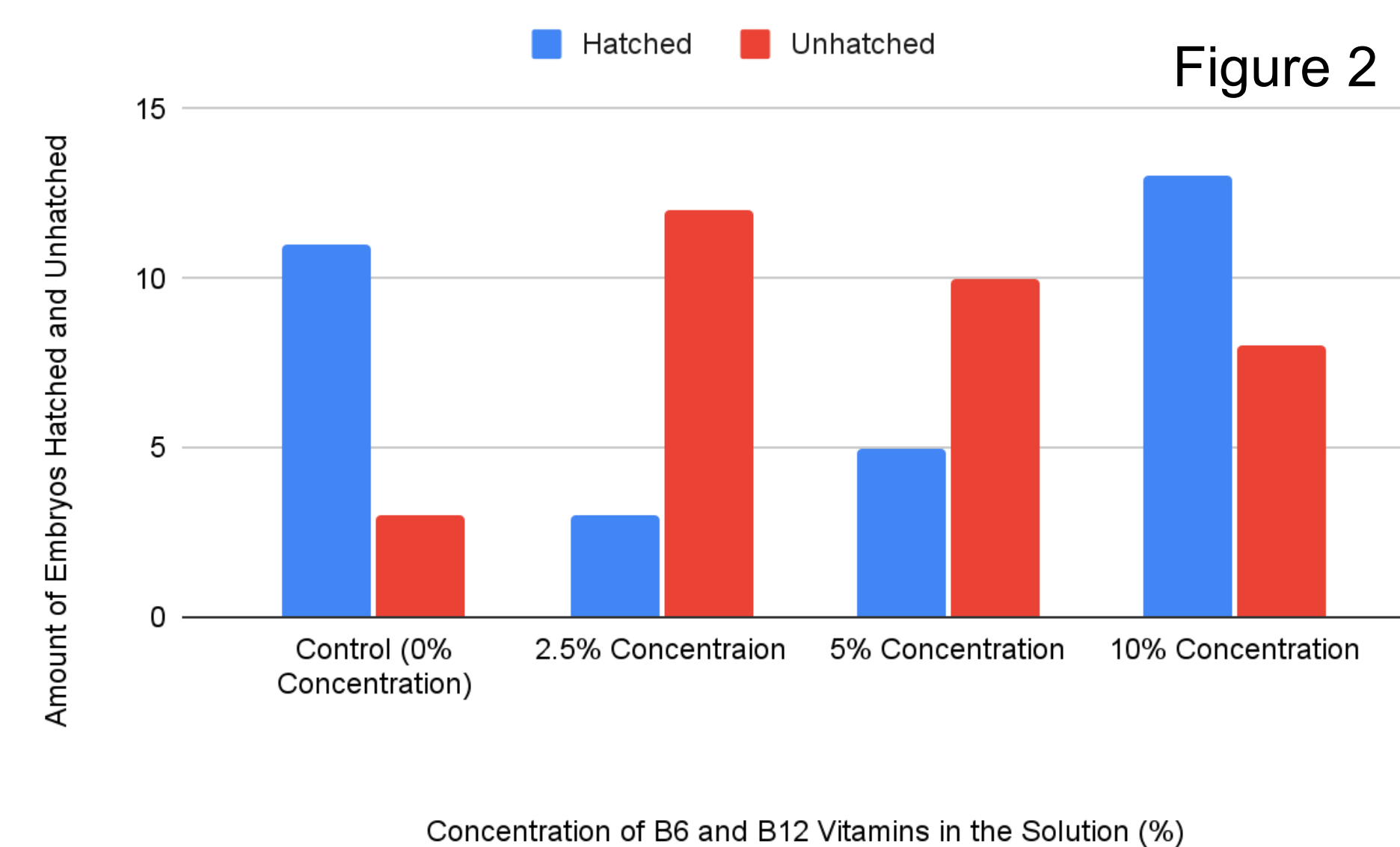
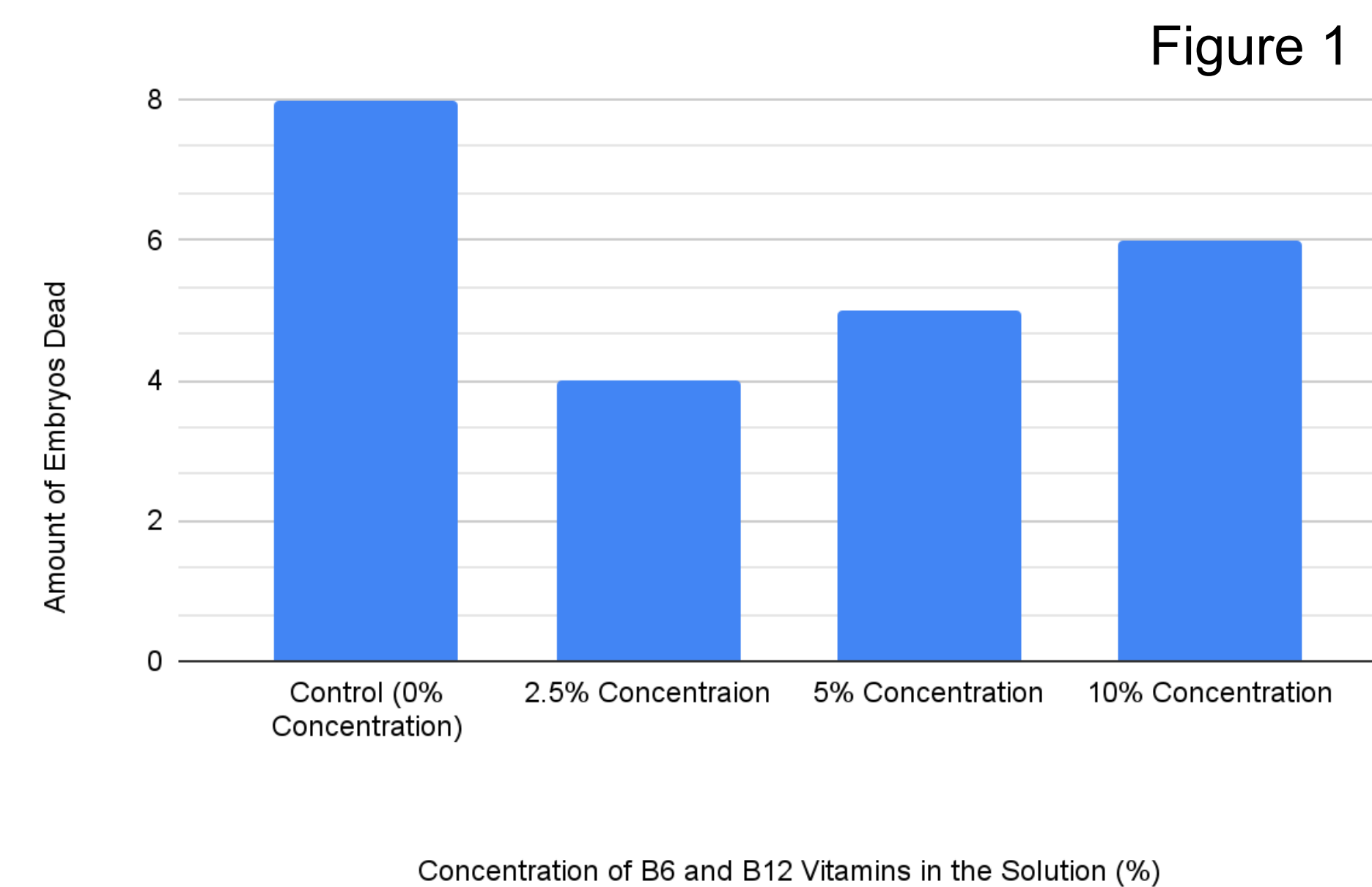
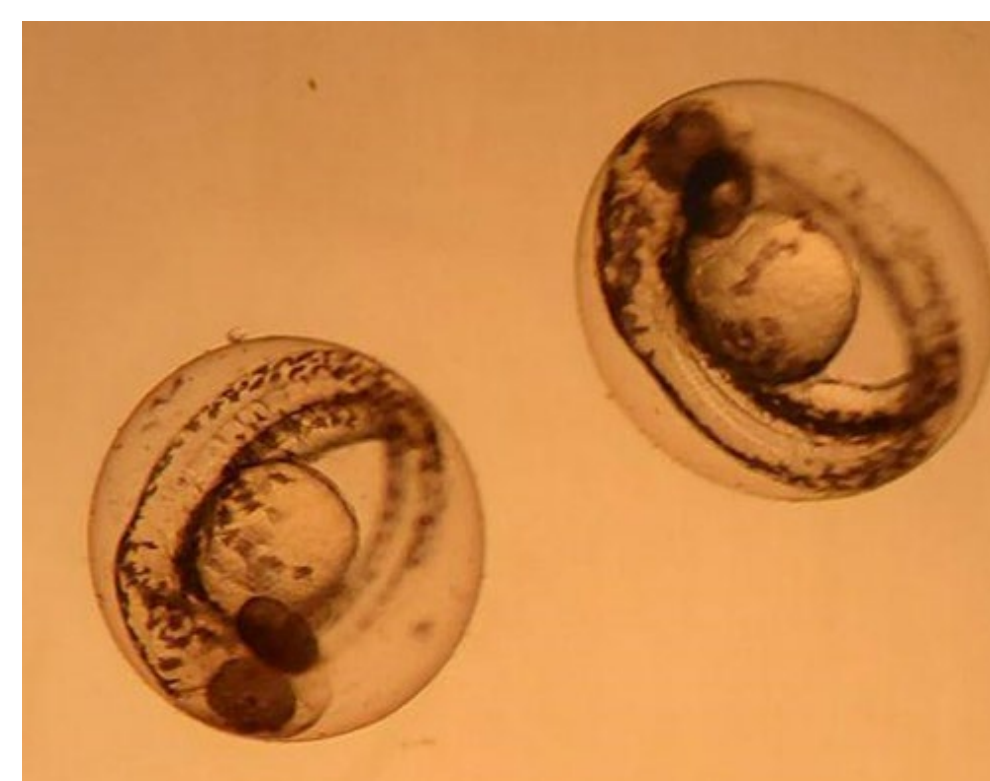


Figure 3



The embryos in the 10% solution with curled tails.

Figure 4



The embryos in the 2.5% solution day three

Discussion

Based upon the results of this experiment, the original hypothesis of the embryonic development of zebrafish in an environment with vitamin B6 and B12 was partially disproved. We found that the vitamins were beneficial to the fish in the lower concentrations. We expected to see healthier and more developed embryos as the concentration of vitamins increased. We also noticed that in the 10% concentration, the tails of the embryos were curled. A source of error that could have contributed to these strange findings is there could have been an inconsistent amount of vitamins across all concentrations. Another source of error could have been that there might not have been the exact same amount of solution in each of the wells in the falcon dish. If we were to repeat this experiment, we could change the dilutions of the concentration so that the vitamins would be more beneficial to the fish. Because of the similarity between zebrafish embryos to human embryos, we can infer that when pregnant women take a large amount of vitamins, it may actually be harmful towards the fetus (2). Based on the deformities that we observed from the zebrafish embryos, this may also affect human fetal development.

References

1. Brezovar, Erin, and Sonia Gude. Developmental Effects of Vitamins on Zebrafish Embryos. SEPA online journal, 2018.
2. Molloy, Anne M., et al. "Effects of Folate and Vitamin B12 Deficiencies during Pregnancy on Fetal, Infant, and Child Development." Food and Nutrition Bulletin, vol. 29, no. 2 Suppl, June 2008, pp. S101-111; discussion S112-115.
3. Brown, Mary J., et al. "Vitamin B6 Deficiency." PubMed, StatPearls Publishing, 2021.
4. Dietrich, Kristin, et al. "Skeletal Biology and Disease Modeling in Zebrafish." Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research, vol. 36, no. 3, Mar. 2021, pp. 436–58.