The Effect of Taurine on the Embryonic Development of Zebrafish Embryos

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Abstract

Taurine is commonly found in energy drinks, meat, shellfish, dairy, and dietary supplements. The main objective of this experiment is to see how Taurine affects embryos. Every day pregnant mothers consume everyday products containing Taurine, such as meat. This experiment's goal is to figure out if this particular ingredient, out of many, in these substances are helping their embryos develop or not. This experiment was done on zebrafish as they are a prime candidate due to their DNA similarity to humans. This information can be applied back to whether or not pregnant mothers should be consuming these everyday products to help develop their embryos or if they are detrimental to them. In the experiment, zebrafish embryos were put into various different solutions containing various dosages of Taurine. The experiment took place over 72 hours with 3 observation periods. The data shows that the mortality rate increases in the Taurine solutions, but the developmental process increases in the embryos. This connects back to whether or not dairy, shellfish, and other products containing Taurine are good for human development in embryo form. This is a basic research study and does not factor in other ingredients in these products, but rather opens a discussion about Taurine consumption while pregnant and how an uptake in products containing taurine may be beneficial to the development of human embryos.

Introduction

Taurine is a chemical that is frequently used in energy drinks such as Red Bull and 5-Hour Energy. The chemical is also naturally found in the human body and is an amino acid that helps with the metabolism process. This experiment will be testing the effect of the addition of Taurine into zebrafish embryos to test if the embryonic development rate will be affected. Embryos may die due to irregularities in their environment; the purpose of this experiment is to test to see if Taurine will improve, not affect, or decrease the quality of the factors in their environment to ultimately affect their development rate. High concentrations of Taurine are in cells of the female human productive system and many believe it to be essential to keeping embryos alive and furthering their development (Choi, 1998). Taurine is known to cause many species to increase fertilization in sperm and increase intracellular organelle activity (Wu, 2011). The Taurine amino acids help to create proteins around the embryo to assist in regulating its metabolism, pH level, and the integrity of cells by acting as osmoregulators (Devreker, 1999). Taurine helps protect embryos from high concentrations of potassium and other substances by acting as an osmolyte to add and remove various substances from the cell as needed, including water (Dumoulin, 1997). All these factors of Taurine help the embryos to grow, survive, and keep their environment regulated--sequentially resulting in a higher survival rate of the embryos.

This experiment can be used as a model for human embryos. The mission of this experiment is to figure out if Taurine is safe for human embryos by not risking human embryos. By using fish

embryos, the results can show how they are affected and can be applied back to human embryos since zebrafish share 70 percent of genes with humans (Bradford, 2017). The results of this experiment could determine if pregnant women should consume or surround themselves with Taurine or products containing Taurine. If the embryos help increase the rate of development of the embryos, it could be beneficial for pregnant women to take Taurine supplements. It is hypothesized that the addition of Taurine into developing embryos enhances the conditions the embryos are in and will further their developmental stage, meaning more embryos will hatch.

Materials and Methods

Materials

- 5 zebrafish per compartment in falcon dish (total of 73)
- 350 mg of Taurine
- 5 flasks
- 4 coffee filters
- 1 funnel
- 4 different colored tapes
- 4 pipettes
- 2 Falcon dishes
- 1 pestle and mortar

Methods

The materials were acquired in an appropriate manner and set aside. 350 mg of Taurine was ground up using the pestle and mortar: one mixture had 200 mg of Taurine, another with 100 mg, another with 50 mg, and one with zero mg of Taurine to act as the control. The different concentrations of Taurine were then mixed with their own 100 mL .2g/L of InstantOcean solutions for three minutes. After the three minutes, the solutions were put through a filtration system using a coffee filter and a funnel until the solutions were fully filtered without any leftover undissolved particles of Taurine. Any leftover and undissolved Taurine from the solutions were discarded. Each solution was then labeled with their respective number of tablets and covered in foil to ensure no unwanted particles would taint the solutions, as well as keep the solutions from evaporating. The pipettes were labeled and then four rows of four compartments of Falcon dishes were filled with three mL of their solution (see Illustration 1). Five embryos were then added to each compartment. The falcon dishes were then left for 24 hours in an incubator that was heated to 28 degrees celsius. After 24 hours, the embryos were then looked at under a microscope where they were observed to see if they had darker pigmentation, movement, or were dead. The number of dead embryos and the number of alive embryos were written down. The dead embryos were promptly removed from each compartment, and any abnormalities in the embryos were photographed and recorded. The living embryos were then observed under a

microscope for their developmental stage, any changes were recorded. This step was repeated once a day for two more days. At the end of the experiment, all of the embryos from the Falcon dishes were removed and the solutions were discarded properly.

Illustration 1

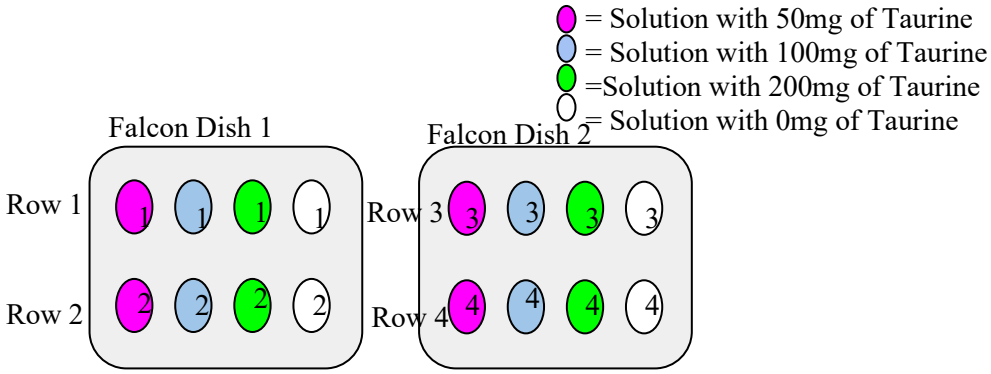

Illustration 1 shows the set up we used for inserting the different solutions into the falcon dishes. As shown, we had four compartments for each different solution across two different falcon dishes with around give embryos in each compartment.

Safety Precautions

- Safe disposal of dead embryos.
- Safe disposal of solutions.
- No cross-contamination of pipettes or solutions.
- Proper disposal of the living embryos at the end of the experiment by the instructor.

Results

The objective of this experiment is to test if Taurine will increase the rate of development in embryos. The embryos were put in different solutions containing 0 mg, 50 mg, 100 mg, or 200 mg of Taurine and were observed to see the developmental stage they were at and how many survived over the course of three days. The independent variable of the experiment conducted was the amount of Taurine added to the solutions that the zebrafish were put into. Oppositional to this, the dependent variable of this experiment was the developmental stage that the zebrafish embryos were at. The controls included the 0 mg of Taurine solution and the ten percent concentration of InstantOcean to make the conditions of the solutions for the embryos stable. These controls provided comparisons for the independent variables to prove the hypothesis that Taurine can, in fact, increase the rate of the development stage of zebrafish embryos.

After this experiment was conducted, the embryos that were in the solutions with Taurine showed to have a higher rate of development. The control zero mg of Taurine solution's embryos had a 36% hatch rate, while the embryos in the 50 mg solution had a 100% hatch rate, the embryos in the 100 mg solution had a 88% hatch rate, and the embryos in the 200 mg solution had a 67% hatch rate. The solution containing 50 mg of Taurine was the most optimal for embryo development as it had the highest success rate. The higher concentrations of Taurine were likely to be too much for the embryos, and while still effective, they likely increased the concentration too much and caused a lower rate of development growth compared to the 50 mg solution. All of the hatch rates for the solutions containing Taurine were near at least twice the rate of the control solution. The mortality rate, however, increased at least 20% in the solutions containing Taurine. The control embryos had a mortality rate of 32%, while the embryos in the 50 mg solution had a mortality rate of 53%, the embryos in the 100 mg had a mortality rate of 50%, and the 200 mg solution embryos had a mortality rate of 63%.

Figure 1

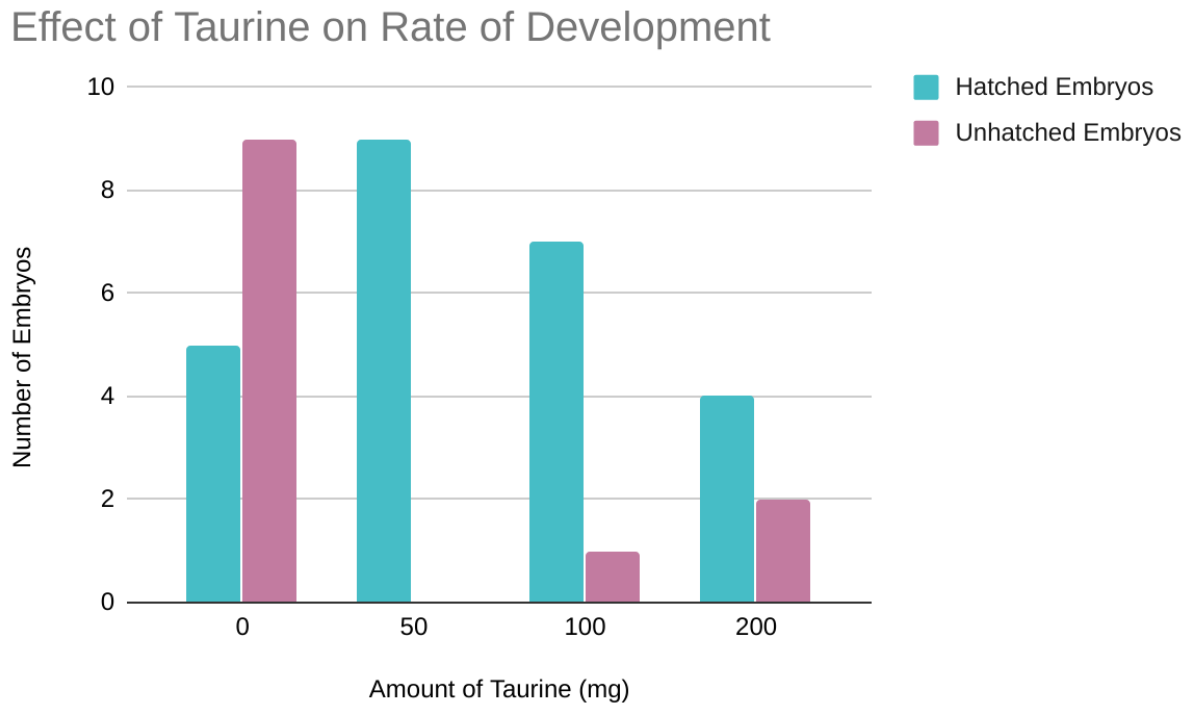

Figure 1 shown above displays the overall count of how many embryos hatched or were stuck in an earlier stage of development on day 3. The data shows that the 50 mg of Taurine was the most successful in hatching embryos with a 100% hatch rate. All of the embryos in the solutions containing Taurine had a higher rate of development compared to the control with 0 mg of Taurine with only a 36% hatch rate.

Figure 2

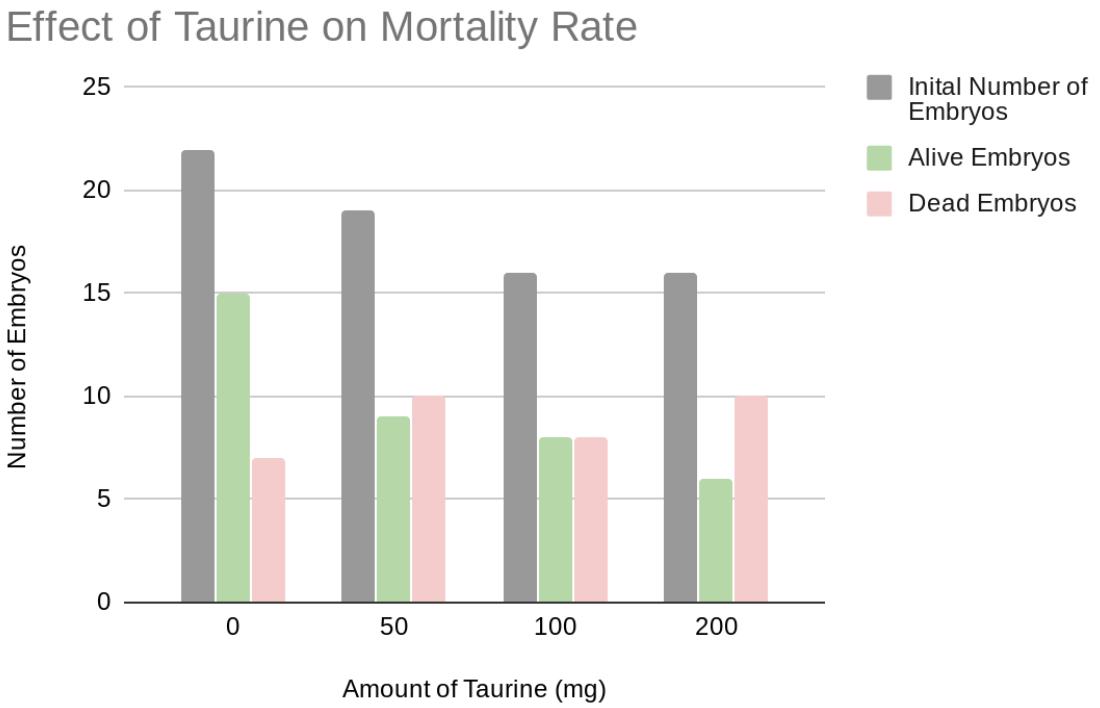

Figure 2 shows the data pertaining to the mortality rate of the embryos based on individual solutions. The grey section shows the total number of embryos in each solution, while compared next to how many embryos died and lived over the course of the experiment. The data is fairly scattered; however, the mortality rate increased for the embryos in the solutions containing Taurine.

Day 1:

Dosage of Taurine	# of Embryos Alive	# of Embryos Dead	Stage of Development
0 mg	15	7	15 Segmentation
50 mg	9	10	9 Segmentation
100 mg	10	6	10 Segmentation
200 mg	7	10	6 Segmentation

Day 2:

Dosage of Taurine	# of Embryos Alive	# of Embryos Dead	Stage of Development
0 mg	15	0	15 Picking up pigmentation
50 mg	9	0	8 Picking up pigmentation 1 Hatchling
100 mg	8	2	8 Picking up pigmentation
200 mg	6	0	2 Hatchlings 4 Picking up pigmentation

Day 3:

Dosage of Taurine	# of Embryos Alive	# of Embryos Dead	Stage of Development
0 mg	14	0	9 Picking up pigmentation 5 Hatchlings
50 mg	9	0	9 Hatchlings
100 mg	8	0	1 Picking Up pigmentation 7 Hatchlings
200 mg	6	0	4 Hatchlings 2 Picking up Pigmentation

Figure 4

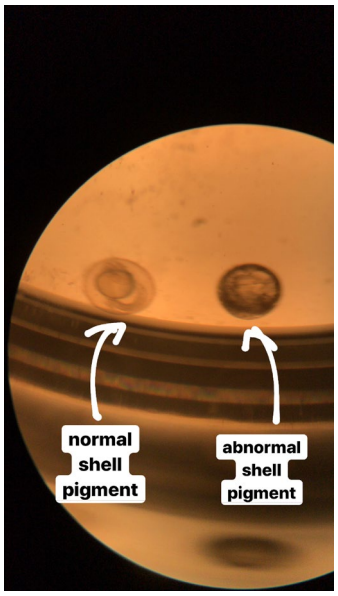

Figure 4 shows an abnormality in pigmentation. During the segmentation stage on the first day of the experiment, the 100- mg treatment of Taurine caused one of the embryos to turn a darker color.

Day 3 Images

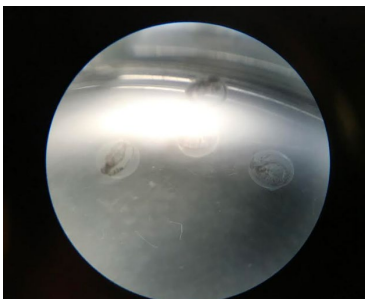

Figure 5

Figure 5 shows the unhatched embryos in the 50 mg Taurine solution during the segmentation period. The eyes and head aren't very defined, therefore, it is in the 6 somite stage in segmentation.

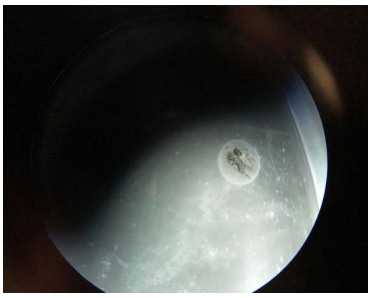

Figure 6

Figure 6 demonstrates an unhatched embryo in the 0 mg Taurine solution during the segmentation period. The eyes are large and defined, meaning they are either in the 10 somite or 14 somite stage in segmentation. This embryo is likely to hatch soon.

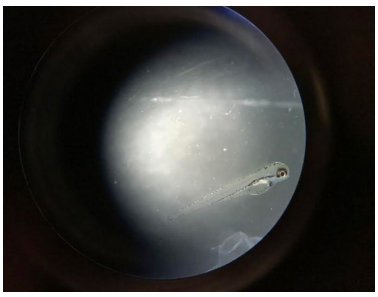

Figure 7

Figure 7 displays a hatched embryo in a 50 mg Taurine solution compartment. As shown, the embryo does not display any abnormalities, making it a healthy embryo.

Discussion

In conclusion, the results were as predicted in the hypothesis. In the control, only five out of the 15 living embryos hatched, while all nine of the living embryos hatched in the 50 mg treatment of Taurine. Although, only four of the eight living embryos in the 200 mg treatment of Taurine hatched, which was less than the 50 mg. High levels of Taurine are beneficial to developing human embryos, but seemingly, there is a limit to how much Taurine is beneficial to the embryos. At some concentrations, they're much more likely to die. This is supported by the fact that 68% of the control fish lived, while only 47% of the 200 mg treatment of Taurine lived. This data suggests that Taurine could potentially increase the mortality rate. This, however, cannot be proven by this data alone as it does not follow a set pattern aside from the trend that Taurine solutions had a higher mortality rate compared to the control. This could mean that uptake in smaller doses of Taurine from sources like dairy, eggs, and meat could be beneficial to embryonic development, but taking extra supplements with extremely high concentrations of Taurine could be deemed dangerous. Since it is known that embryos die to abnormalities in their environment, it is expected for them to have a higher mortality rate.

One abnormality that we saw in action was in Figure 4. The embryo is seen as having a dark pigmentation compared to all of the other embryos. One of the main reasons that pigmentation colors of embryos change is due to excess proteins. This occurrence could be explained by the addition of Taurine, as extra amino acids could affect its cells' pigmentation by the addition of amino acids, the building blocks of proteins, to its pre-existing proteins. The pigmentation does

not affect the embryo, but may instead indicate a change in genes regulating the melanin production for the gene, which will later affect the pigmentation of the zebrafish. This abnormality could be due to the addition or Taurine or could be a different external or internal factor.

If this experiment was to be done again, it would be advantageous to make the Taurine solutions less concentrated, sticking to around 50 mg or less, since the embryos in the 50 mg solution hatched the quickest. The limitations in these experiments include not having a second experiment on a different date repeated to guarantee the same results. These results could mean that in low concentrations, Taurine can be beneficial to embryos. While 50 mg seems to be the correct dosage for five zebrafish embryos, human embryos are much bigger and may require more Taurine. The recommended dosage for a human female of Taurine is 500-2000 mg per day. However, toxicity levels allow for 3000 mg per day to be taken without any side effects (Curran, 2017). This means that if a human woman were to increase her dairy, seafood, and other Taurine-containing products intake, up to 2500 mg-2700 mg per day, then there is a chance that her embryonic development could be supported by Taurine.

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