The Effect of Ethanol on Zebrafish Embryonic Development

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Abstract:

Approximately 10% of women reported drinking while being pregnant. The ethanol causes deformities in human babies and zebrafish as well. This is important to know this because many children have the risk of getting defects. The purpose of this experiment was made to show what could happen to the child if a mother drinks while she is pregnant. In this experiment, zebrafish were exposed to ethanol (0 mM, 30 mM, 100 mM, and 300 mM) and the zebrafish were monitored for five days. The number of eye defects were recorded every 24 hours.

This experiment supported our hypothesis; if zebrafish are exposed to a higher concentration of ethanol, then the ones exposed to a higher concentration will show more defects than the ones with lower levels of ethanol. This is because ethanol in humans shows a higher increase in birth defects. The zebrafish exposed to ethanol had a higher deformity rate than embryos that were not exposed to ethanol. Results showed six eye defects meaning the fish had only one eye, four of those were in 300 mM which supports the hypothesis.

As the ethanol levels increase more defects were present, which supported research that stated zebrafish exposed to ethanol are more likely to have developmental problems. The research in this experiment supports the point that exposure to alcohol in fetuses cause defects in babies, the only differences are the types of defects. Zebrafish defects made the fish only have one eye however, in human babies the defects would be more related to body parts not concerning eyes such as lips, ears, and noses.

Introduction:

One in ten women reported drinking while being pregnant which caused ethanol exposure to the fetus as seen in the article (CDC, 2015) . Zebrafish have very similar genetic coding to humans. Furthermore, zebrafish have a clear embryo to see what's going during embryonic development while doing experiments which makes them an ideal model organism, to use to draw comparisons to human fetal development. In humans, if a mother is pregnant with a fetus/baby and drinks alcohol the baby also drinks it. According to Pregnancy and Alcohol (2012), alcohol can cause a range of symptoms connected to the illness known as Fetal Alcohol Spectrum Disorder (FASD). These symptoms range from behavioral dysfunctions, problems with the heart, kidneys, and/or bones, birth deformities, and growth development slowness. (Pregnancy and Alcohol, 2012). This is important because zebrafish embryos are similar to human embryos, whatever might happen to zebrafish embryos may have some similar effects to human embryos. Drinking while pregnant can have other more severe consequences, in the text (Development Timeline of Alcohol-induced Birth Defects) it states, “Those developmental deviations can result in a range of birth defects or may completely arrest the pregnancy.” This proves that there are heavier consequences while drinking when you’re pregnant, completely arresting the pregnancy (killing the offspring/embryo) would be one of them because you're losing the baby. In order to achieve FASD, you do need a good amount of alcohol in your body as one article states that, “With very repetitive doses there is a 6-10% chance of the fetus developing the fetal alcohol syndrome manifested by prenatal and postnatal growth deficiency.” (Alcohol Abuse in Pregnant Women, 2010). This concludes that you would have to have about 5-7 drinks/ shots of alcohol a week for the fetus to develop FASD. The purpose of this experiment was to add ethanol to developing zebrafish embryos and draw possible correlation of the effects on human development if the mother consumes alcohol while pregnant. The

experimental hypothesis was, if zebrafish are exposed to a higher concentration of ethanol, then the ones exposed to a higher concentration will show more defects than the ones with lower levels of ethanol because ethanol in humans shows a larger increase in birth defects.

Materials and Methods:

- Solutions of ethanol and a control group
    - 0.0 mM ethanol (control group)
    - 30 mM ethanol
    - 100 mM ethanol
    - 300 mM ethanol
- Sharpie (for label)
- Painters tape (for label)
- 28.5 ºC Incubator
- Tray with 12 wells and a cover
- Beaker (50 mL) for dead embryos and liquid disposal
- Pipette 1.5 ml for transferring dead eggs and liquid into the beaker for disposal
- Pipette 1 mL to measure how much ethanol solution is put into one well
- Dissecting microscope
- IPad - camera and recording sheet
- Recording sheet for qualitative and quantitative data
- 40 embryos (10 for each top four wells)

Day1: Get a tray with a lid along with a roll of painters tape and a black sharpie. From then put three pieces of painters tape from side to side than label the top the amounts of ethanol (0.0 mM, 30 mM, 100 mM, 30 0mM) and “Ethanol” as the subject on the top. Next, take 10 embryos from the tank with the bigger sized pipette and add them to each well, until there is a count of 40 in all. Then with the 1.5 ml pipette drain the excess liquid making sure to not take out any of the embryos and recounting them all after to make sure there is 10 embryos in each well. From there on add the solutions to each of the labeled wells making sure the control group (0.0 mM) comes first. Take pictures under the microscopes of each well and place them in a document

and label how many died in each well and any additional information.Fish were determined dead after they had no movement or not heartbeat which can be seen under the microscope. After this put them on the cart which will transport them to the incubator (28.5 degrees C) later on.

Day2-4: Observe embryos and record qualitative and quantitative data. Take pictures daily and label each photo dead, alive, or other observations (such as deformities). Carefully take out solutions using 1.5 ml pipette, without removing any healthy embryos and/or fish. Replace solutions in the correct wells making sure that everything is correct. Then clean up and place embryos back on cart to go in incubator.

Day 5: After, take qualitative and quantitative data for the last day, than take pictures of each and label the amount of dead embryos/ live fish and alive ones, and other observations (such as deformities). When done, take a pipette that is wider suck the fish up gently and put the alive fish in the fishtank. Then take the rest of the solution and dead embryos and dispose of it properly. The data was finally measured after the last day because some embryos were not hatched. So in order to get as many numbers possible, all the eggs had to be hatched.

Caution: Keep the ethanol solutions away from your mouth as they may harm your body if it is used internally, make sure to use gloves. A chi square analysis was completed on the data to ensure statistical significance

Results:

The purpose of this experiment was to see what effects ethanol has on zebrafish and how it compares to the fetus in the womb of a human. The dependent variable was the defects and the number of defects in each fish. The independent variable is the amount of each solution (0.0 mM, 30 mM, 100 mM, and 300 mM). The control group was 0.0 mM which contained no ethanol. The controlled variables in this experiment were also the good size, incubator temperature, and the amount of the solution. The fish that were in the (0.0 mM) showed no eye defects along with (30 mM) as seen in the pictures below (figures 3-4). However, solutions 100 mM and 300mM showed in total six eye defects, (100 mM) had two eye deformities, and 300mM had four eye deformities. As seen in figures 5-6 comparing the eye deformities and what they look like compared to the fish without eye deformity. There was a trend found in the data when the ethanol levels are increased, so is the number of eye deformities. As seen in the chi-square analysis the degree of freedom used in the Chi-square analysis was 3 and the final chi-square value was 21.04. The critical value was 7.52 and the final number concluded was well above this number. Therefore rejecting the null hypothesis. The Chi-square analysis performed on this data shows that the data collected in this experiment cannot be by chance and the ethanol did affect the number of deformities seen in the zebrafish.

Treatment (Ethanol)	Eye Defects	No Eye Defects	Total for Rows
0.0 Control Group	0	9	9
30 mM	0	7	7
100 mM	2	5	7
300 mM	4	3	7
Total for Columns	6	24	Total for table 30

Table 1: This table shows the number of eye defects caused by ethanol amounts (0.0mM, 30mM, 100mM, 300mM) compared to no eye defects.

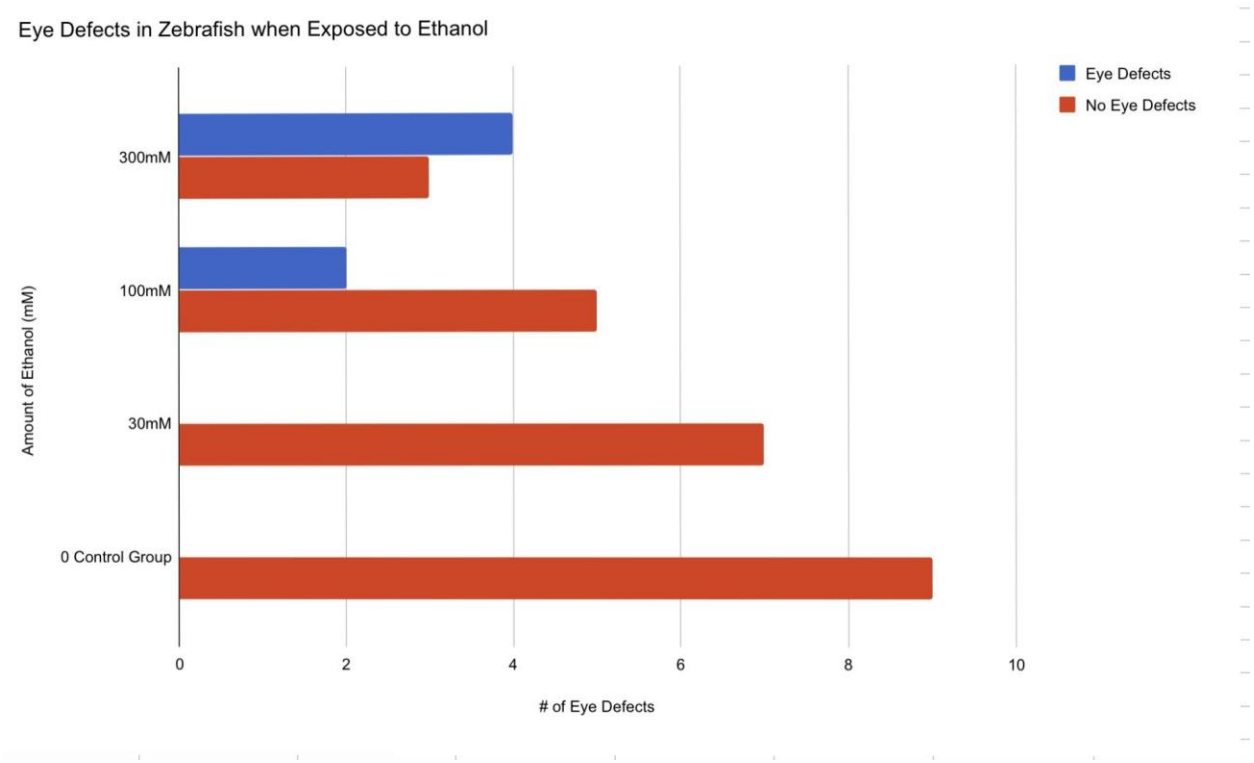
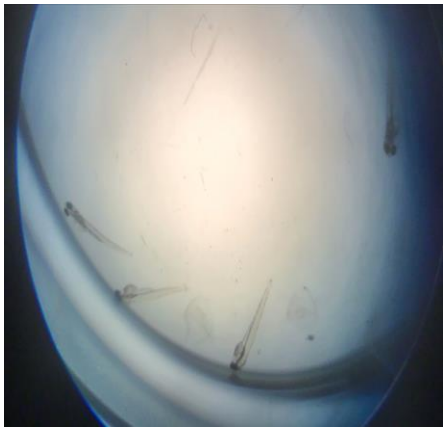


Figure 2: The chart above is comparing number or eye defects to the number of fish that did not have eye defects depending on the amount of ethanol. As the amount of ethanol increased the numbers of eye defects did as well.



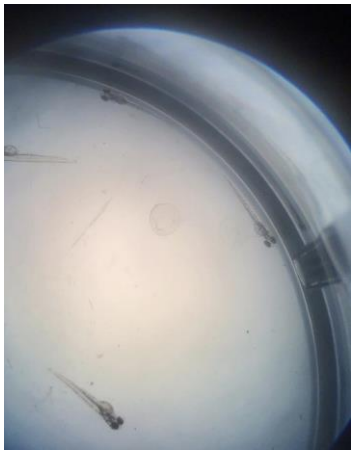


Figure 3-4: Pictures of 0.0 mM and 30 mM, each fish with two eyes and no defects in their eyes.

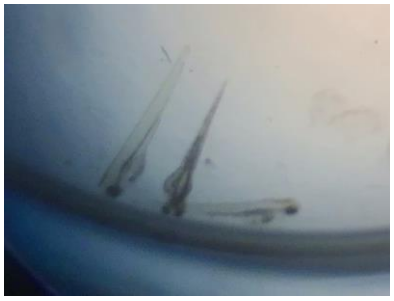


Figure 5-6: Pictures of the fish in 100 mM and 300 mM with the one eye deformities. The picture on the right side is a fish with the eye deformity compared to a fish without an eye deformities.

Discussion:

The results of the research support the hypothesis because, if zebrafish are exposed to a higher concentration of ethanol, then the ones affected with a higher concentration will show more defects than the ones with lower levels of ethanol. In the end, 30 fish alive and 6 out of the 30 had only one eye. This supports the hypothesis because in the higher amounts of ethanol the number of eye defects increased. In 100 mM there were 2 defects in total in 300 mM there were 4 defects. Some trends found in the data are the number of eye defects increased as the ethanol liquids increased. For example, in the

data, in the solutions 0.0 mM and 30 mM had no eye defects but 100 mM had 2 and 300 mM had 4 eye defects.

One way to improve this experiment is to let the zebrafish sit in the ethanol solutions longer. Also, there could be more fish embryos for more exact results rather than the number this experiment had. For example, having 80 fish embryos instead of 40 to have more results. An unanswered area is, could the ethanol affect the death total in zebrafish embryos. Also, if the ethanol affects the zebrafish in later growth, will the ethanol affect the offspring. For example, if a mother had ethanol abuse in the womb would their child be affected too?

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