The Effect of Alcohol on the Mortality Rate of Zebrafish Embryos

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Abstract

This experiment was conducted to investigate the effect of alcohol on embryonic development. One in ten women reported drinking alcohol while pregnant, causing alcohol exposure for their babies (Center for Disease Control and Prevention, 2015). The experiment supported the following hypothesis: If the zebrafish embryos are exposed to either no alcohol or alcohol, the embryos exposed to alcohol will have the highest mortality rate, because the alcohol will cause the organs of the embryos to malfunction, which ultimately will lead to death.

Zebrafish eggs were placed in alcohol concentrations of 0.00%, 0.06%, and 0.3%. These solutions were picked to avoid having both the experimental alcohol concentrations completely kill the embryos and to resemble possible concentrations of alcohol exposure for human embryos (Petering, Berg, Tomasiewicz, et al., 2018, 39). The embryos were monitored for five days and the number of dead embryos in each group was recorded after each 24 hour period.

According to the statistical significant experiment, the zebrafish alcohol-exposed embryos had a higher mortality rate than the embryos not exposed to alcohol ($p=0.0498$). The alcohol caused abnormalities in the organs of the embryos that lead to death. Because of the similar anatomy of zebrafish embryos and human embryos, the results of the experiment involving zebrafish can correlate to human embryos also having a higher mortality rate and developmental problems when exposed to alcohol (Burke, 2016). Previous experiments have

also found that the zebrafish exposed to alcohol had many developmental problems (Sylvain, Brewster, Ali, 2010).

Introduction

Zebrafish as Model Organisms

Zebrafish are frequently used in experiments because of many factors. Zebrafish are model organisms, or non-human organisms that are widely studied to obtain knowledge on biological processes (Kinith, Mahesh, Panwar, 2013). Zebrafish embryos have transparent larvae stages, which allows for the developing embryos to be examined throughout all the stages of development (Burke, 2016). The death of an embryo can be easily seen from the color change inside the egg (Burke, 2016). This makes it easy to record data throughout their development. Zebrafish are cheap to maintain, have hundreds of offsprings in a short amount of time and they grow extremely fast (Burke, 2016). These factors make zebrafish a great organism to study in a small space, such as a classroom, and allowed for a large sample size to be used in a small space. Additionally, zebrafish share 70% of their genes with humans, and the zebrafish also have similar internal features to humans (Burke, 2016). Because of the similarity between the zebrafish and humans, the results of many scientific experiments using zebrafish can directly correlate to the results that would have been produced if human embryos had been used in a similar experiment (Burke, 2016). Many factors and traits of zebrafish cause them to be great model organisms to use in experiments (Burke, 2016).

Alcohol

Alcohol, especially ethanol, is found in many drinks that are commonly consumed by adults. Alcohol is an organic molecule that is made of hydrogen, carbon, and oxygen (“What is

alcohol", 2019). The chemical structure of alcohol is C_2H_5OH, which means it has a polar hydroxyl group ("What is alcohol", 2019). Alcohol is also soluble in water and small in size, which allows alcohol to enter the bloodstream and move throughout the body ("What is alcohol", 2019). The alcohol then leaves the capillaries and enters tissue, like the brain, because of the gradient difference between the capillaries and tissue in the body ("Where does alcohol go", 2019).

There is a blood barrier that surrounds the brain, which is supposed to prevent harmful substances from entering the brain; however, alcohol is small and slightly lipophilic, so it can pass the blood-barrier and enter the brain ("Where does alcohol go", 2019). Because alcohol can enter the brain, it has the opportunity to intoxicate the body ("Where does alcohol go", 2019). Females are at a higher risk for alcohol intoxication when drinking the same amount of alcohol as a male. This is due to the lower water concentration in the female body, which causes a higher blood alcohol centration ("Gender differences in alcohol", 2019). Many adults drink alcohol (Center for disease control and prevention, 2015). In 2016, the average amount of alcohol consumed by a US citizen was 2.35 gallons a year ("Alcohol consumption per capita", 2016). An astonishing 10% of pregnant women report drinking in the last 30 days (Center for disease control and prevention, 2015). This means that at least one in ten fetuses are exposed to alcohol while developing.

Although many people do not think drinking alcohol will harm them, drinking alcohol while pregnant has been found to lead to many problems for the fetus (“What are fetal alcohol spectrum disorders”, 2019). These problems are known as Fetal Alcohol Syndrome (FAS) and can occur when a mother consumes alcohol while pregnant (“What are fetal alcohol spectrum disorders”, 2019). It can occur from drinking during any stage of pregnancy (“What are fetal alcohol spectrum disorders”, 2019). Common effects of Fetal Alcohol Syndrome include facial and physical abnormalities, harmed growth, and cognitive abnormalities (“What are fetal alcohol spectrum disorders”, 2019). Many babies with FAS have facial abnormalities including a short nose, a flat midface, a thin upper lip and an indistinct philtrum (“What are fetal alcohol spectrum disorders”, 2019). Many also have insufficient brain growth, organ development failure, and cognitive abnormalities (“What are fetal alcohol spectrum disorders”, 2019). Alcohol causes organ and brain damage for fetuses because of its chemical structure and properties (“What is alcohol”, 2019). The polar hydroxyl group of alcohol has the ability to create reactive oxygen species(ROS) when the oxygen in the alcohol pairs to an unpaired electron (“How does alcohol damage the fetus”, 2019). The molecule is unstable and steals electrons from DNA, proteins, and lipids (“How does alcohol damage the fetus”, 2019). This causes damage and changes to the molecular structure of many cellular molecules within the body of the fetus (“How does alcohol damage the fetus”, 2019). Because the brain uses more oxygen compared to any other part of the body, the brain is most affected by the reactive oxygen species (“How does alcohol damage the fetus”, 2019).

Many other species, including mice and zebrafish, have been exposed to alcohol while developing so that scientists could study them (“What are fetal alcohol spectrum disorders”, 2019). A study scientists completed showed that when zebrafish embryos are exposed to alcohol, they do in fact have abnormal motor neurons and muscle fibers (Sylvain, Brewster, Ali, 2010). The fish exposed to alcohol had an abnormal response when touched (Sylvain, Brewster, Ali, 2010). The zebrafish embryos exposed to alcohol did not move when touched, but normal zebrafish embryos did (Sylvain, Brewster, Ali, 2010). Many other scientists have conducted experiments in which they exposed zebrafish to alcohol. Another scientist found that the zebrafish exposed to the alcohol had genetic mutations and withdrawal symptoms (Gerlai, Chatterjee, Pereira, et al, 2009). When mice were exposed to alcohol while developing, the mice had facial deformities (“What are fetal alcohol spectrum disorders”, 2019). The finding in these experiments using both zebrafish and mice can directly correlate to a pregnant woman drinking while pregnant. When human fetuses, or homo sapien fetuses, are exposed to alcohol while developing, they are affected in a similar way to zebrafish and mice. Studies on animals similar to humans, like zebrafish, can show whether or not the consumption of alcohol by pregnant women is affecting the development of the fetus, without exposing human fetuses to alcohol.

The Investigation

In this investigation, zebrafish, or Danio Rerio, were exposed to alcohol. One group of fish was placed into a solution that contained no alcohol, one group was placed into a solution that contained 0.06% alcohol, and one group was placed into a solution that contained 0.3% alcohol. The zebrafish were placed in the solution 24 hours post fertilization and were removed 96 hours post fertilization. After each 24 hour period, the dead fish were disposed of and the number of fish alive was recorded. This experiment was designed to answer the following research question: Does alcohol adversely affect embryonic development and mortality rate? This experiment is designed so that the zebrafish could represent a human, in order to see whether or not alcohol could be harmful to a developing human fetus.

Hypothesis

If the zebrafish embryos are exposed to 0%, 0.06%, and 0.3% concentrations of alcohol, then the embryos exposed the 0.3% alcohol would have the highest mortality rate, then the embryos exposed to 0.06% alcohol would have the next highest mortality rate, and lastly the embryos not exposed to alcohol would have the lowest mortality rate, because the alcohol would affect the development of the embryos to a point where the organs of the embryos would no longer function, causing death.

Materials and Methods

Participants

This experiment was completed by two freshmen at Greendale High School. Both participants are part of the honors biology course. The University of Wisconsin-Milwaukee was also involved and supported the experiment. They provided the zebrafish eggs and some of the supplies. The procedure was also based off of a sample procedure provided by UW-Milwaukee. Eighty-eight zebrafish embryos were used to conduct this experiment. They were stored at 28.5^{o}C in the incubator for around 23.5 hours per day for the five day period (Petering, Berg, Tomasiewicz, et al., 2018, 39).

Materials

Many materials were used to complete this experiment in a precise and repeatable manner. Many materials were used including these: around ninety zebrafish eggs, a microscope, a well plate, instant ocean solution, two different sized micropipettes, a sharpie, an incubator, two alcohol solutions(0.06% and 0.3%), a waste beaker, and a phone to take photos. Safety goggles were also worn for safety precautions throughout the experiment to prevent alcohol or waste solution from getting into people’s eyes.

Design

In order to keep this experiment reliable, repeatable, and trustworthy, many groups and variables were used. In the experiment, the independent variable was concentration(%) of alcohol in the solution the zebrafish were placed into. The independent variable varies from 0% alcohol, 0.06% alcohol and 0.3% alcohol. There were 12 wells total, 4 for each alcohol concentration. Each well had an average of 7 zebrafish in them at the beginning. Each treatment was repeated 4 times. The dependent variable was the number of fish alive. It was recorded for each of the twelve wells, for each 24 hours period between 24 hours post fertilization and 96 hours post fertilization. In the experiment, the sample size was eighty-eight. The experiment was designed to have a control group and two different experimental groups. The control group was not exposed to alcohol and was only exposed to the instant ocean solution. One of the experimental groups was exposed to a 0.06% alcohol concentration, and the other experimental group was exposed to 0.3% alcohol. Throughout the experiment, data was collected for the number of embryos alive in each well. After the experiment was conducted, the data from the control group and the data from the 0.06% alcohol-exposed group was entered into an online website called GraphPad. With the data, a t-test was run.

Procedure

The zebrafish were bred at UWM and brought the eggs to Greendale High School so that the eggs could be used for experimentation. Around 24 hours post fertilization, the eggs received their first treatment. First, a well plate was obtained. Then, the well plate was labeled

with the participant's names and the alcohol concentration that each row would contain. The correct alcohol concentration of 0.06% and 0.3% were created. Around ten zebrafish eggs were then placed into each of the twelve wells using a micropipette. Using a microscope, each zebrafish embryo was inspected to see whether or not it was alive. The eggs with a dark, fuzzy center were declared dead and then taken out of the well plate and placed into the waste beaker. Following this, the number of living embryos in each well was recorded. Using a fine tip micropipette, the liquid in each well was extracted and put into the waste beaker. The liquid was replaced with the correct alcohol concentration. The four wells in row A were the control group, so these wells were filled with an alcohol-free solution. The zebrafish embryos in row B were placed in a solution with an alcohol concentration of 0.06%, and the embryos in row C were in a solution with a 0.3% alcohol concentration. Finally, the well plate that contained the embryos was placed into an incubator at 28.5^{o}C (Petering, Berg, Tomasiewicz, et al., 2018, 39). At 48 hours post fertilization and then again at 72 hours post fertilization, the embryos were examined under a microscope. The dead embryos were discarded into the waste beaker and the number of alive and hatched embryos was recorded. Photos of the embryos were also taken and qualitative data was recorded. Then, the solution the embryos were in was taken out using a micropipette and replaced with a fresh solution of the exact same alcohol concentration (Petering, Berg, Tomasiewicz, et al., 2018, 39). On the final day, 96 hours post fertilization, the number of alive and hatched embryos were counted and qualitative data was recorded. The zebrafish were then discarded.

Results

Experimental Design

Throughout this experiment, many variables and experimental groups were used in a way that kept the experiment reliable and repeatable. The independent variable was the concentration(%) of alcohol for the solution the zebrafish were placed into. The independent variable varied from 0% alcohol, 0.06% alcohol and 0.3% alcohol. The dependent variable was the number of fish alive. It was recorded for each of the twelve wells, for each 24 hours period between 24 hours post fertilization and 96 hours post fertilization. This allowed conclusions to be drawn about the effect of alcohol on the mortality rate of the embryos. The experiment was designed to have a control group and two different experimental groups. The control group was not exposed to alcohol but instead was only exposed to the instant ocean solution. One of the experimental groups was exposed to a 0.06% alcohol concentration, and the other experimental group was exposed to 0.3% alcohol concentration. Many constants remained the same throughout the experiment. The number of well receiving each treatment was constant and was four for each of the three treatments. The amount of time and the timeframe each zebrafish was exposed to alcohol remained the same.

Summary of Results

The data from the experiment showed that the mortality rate of the alcohol-exposed embryos was significantly higher than the mortality rate of the control embryos ($p=0.0498$). The independent variable, or the concentration of alcohol to embryos were exposed to, significantly

affect the dependent variable, which was the mortality rate of the embryos. At 96 hours post fertilization, 57.14% of the zebrafish that were alive 24 hours post fertilization for the control group were still alive. In contrast, only 43.33% of the zebrafish exposed to 0.06% alcohol were still alive and 50% of the zebrafish exposed to 0.3% alcohol were still alive. The dependent variable, the number of zebrafish alive at each 24 hour period, was recorded and used to calculate the percent of zebrafish embryos still alive. Almost an equal number of zebrafish died from 24 hours post fertilization to 48 hours post fertilization for all the group. When looking at the zebrafish still alive at 48 hours post fertilization and then calculating the percent of zebrafish that died from 48 hours post fertilization to 96 hours post fertilization, a major trend was seen. Exactly 69.5% of the zebrafish in the control group remaining at 48 hours post fertilization were still alive at 96 hours post fertilization.

On the other hand, only 58.3% of the zebrafish alive at 48 hours post fertilization in the group exposed to 0.06% alcohol were still alive 96 hours post fertilization. Additionally, the numbers of hatched fish and the timeframe in which they hatched differed from the control to the experimental group. For the control group, nine zebrafish hatched from 72 hours post fertilization to 96 hours post fertilization. Two zebrafish exposed to 0.06% alcohol hatched between 48 and 72 hours post fertilization and only three more zebrafish hatched between 72 hours post fertilization and 96 hours post fertilization. Recorded observations showed that the zebrafish in both experimental groups had curved spines, and more embryos were not able to fully hatch.

Tables and Figures

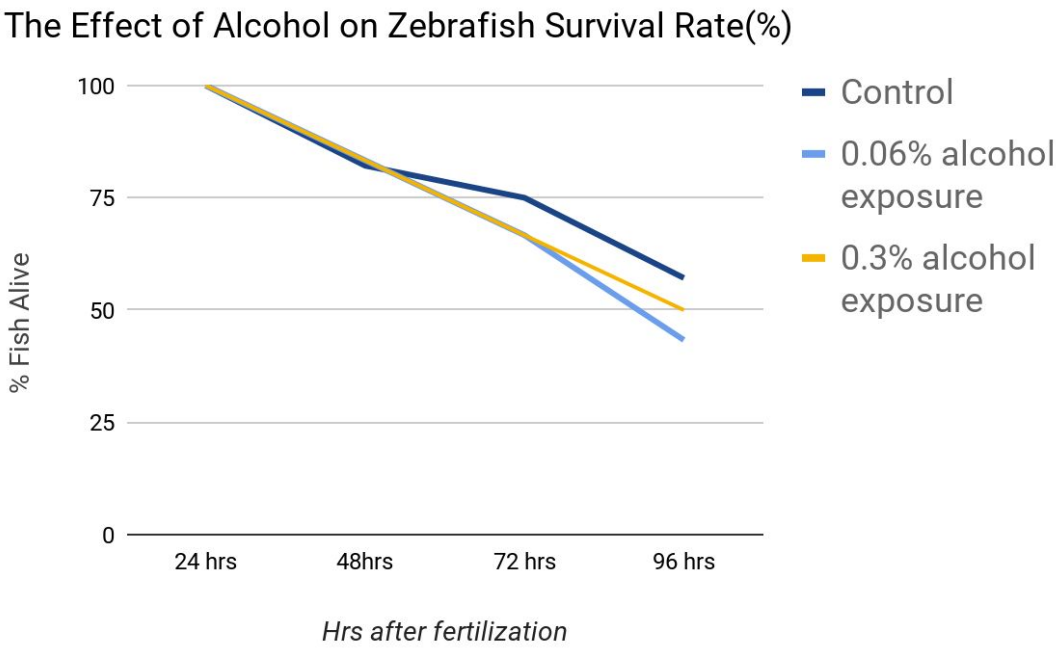


Figure 1:
Trend Statement: The zebrafish embryos exposed to the alcohol experienced higher mortality rates than the control group.

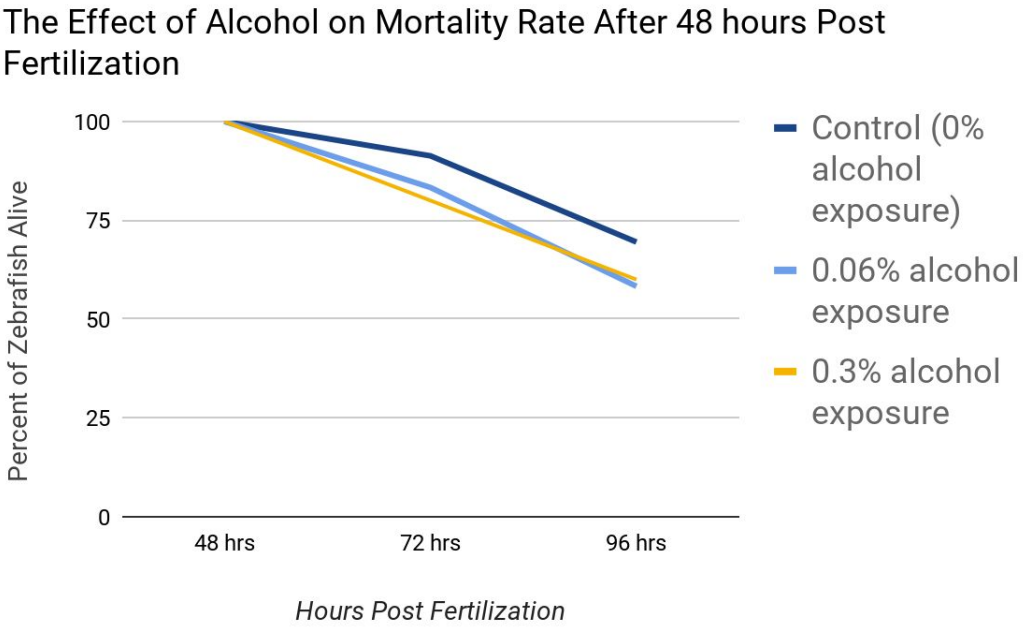


Figure 2:

Trend Statement: The zebrafish embryos exposed to the alcohol experienced a higher mortality rate than the control fish starting 48 hours post fertilization.

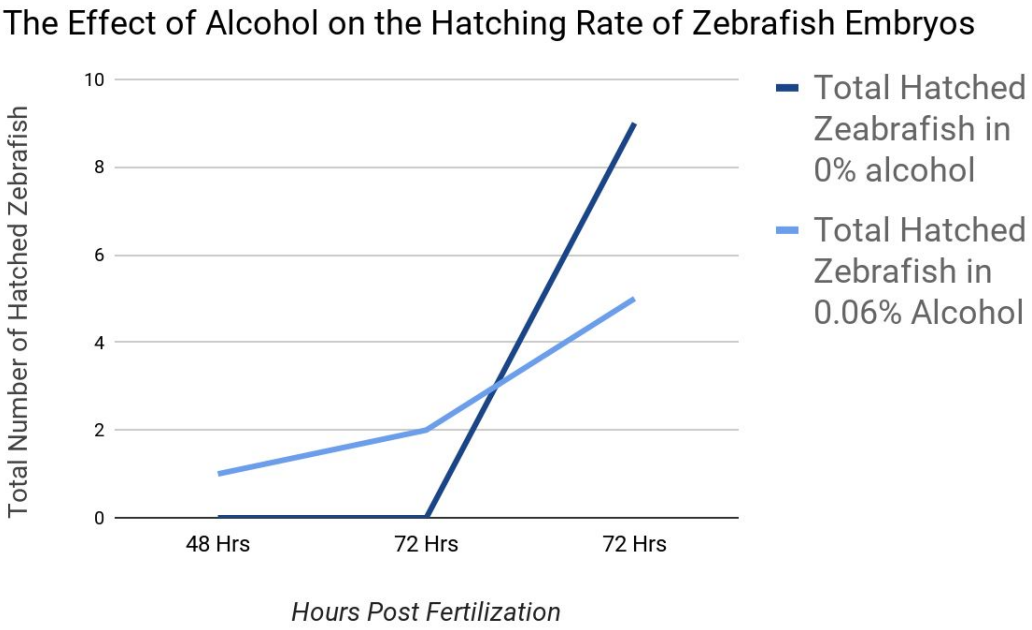
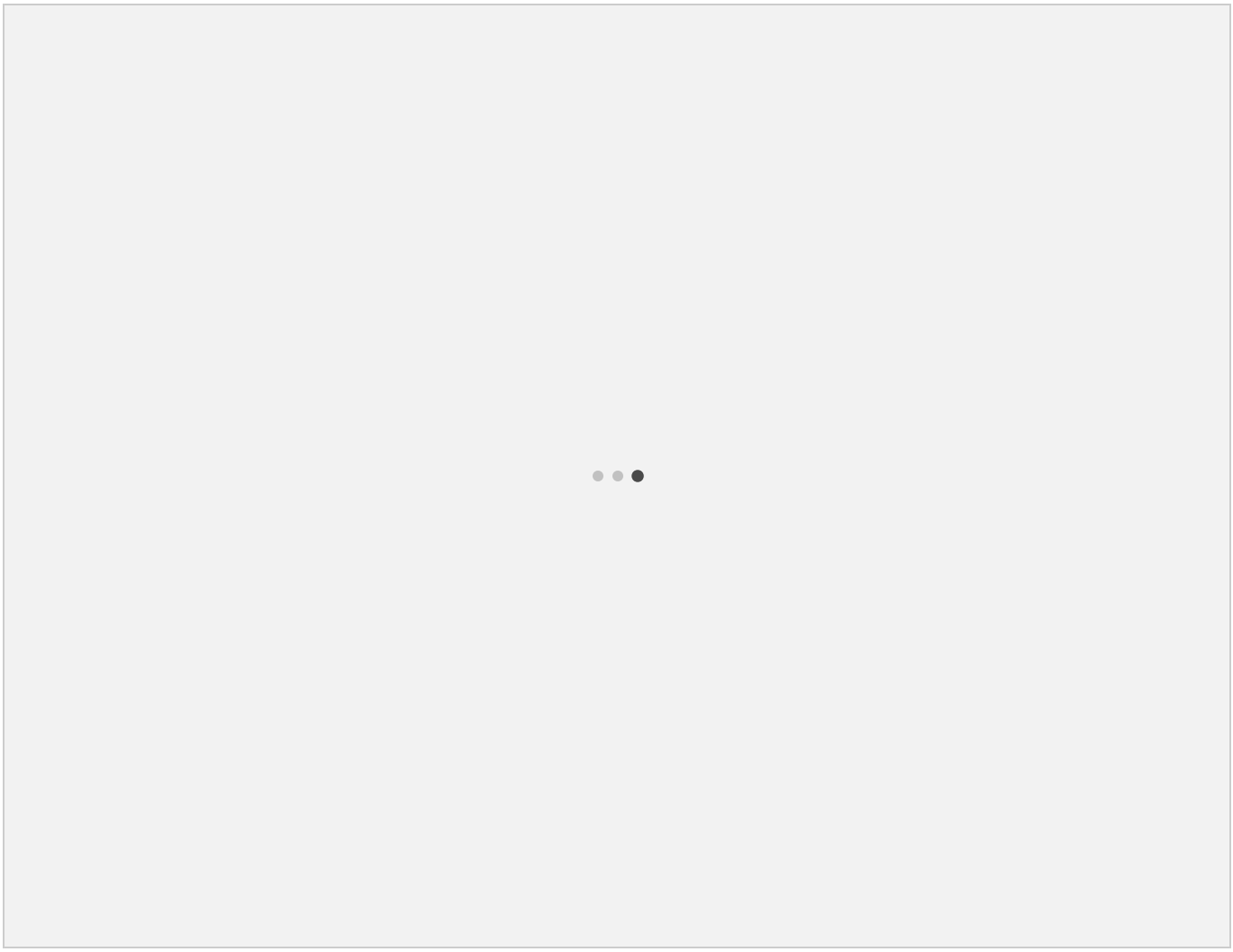


Figure 3:
Trend Statement: Fewer zebrafish embryos exposed to 0.06% alcohol hatched than the control group. On average, the eggs that hatched from the experimental group hatched sooner than those from the control group.

	Control Group No alcohol exposure- Well A1	**Experimental Group 0.06% Alcohol Exposure- Well B1**
24 hours post fertilization	**7**	**5**
48 hours post fertilization	**6**	**5**
72 hours post fertilization	**6**	**1**
96 hours post fertilization	**5**	**1**

Figure 4:

Trend Statement: Many more zebrafish in the experimental group died than the zebrafish in the control group.



Statistical Findings:

To prove that the data from the experiment was statistically significant, the website GraphPad was used to run a t-test. A t-test is an inferential statistic that compares two groups of data. For this experiment, the control (0.0% alcohol exposure) and an experimental group (0.06% alcohol exposure) were compared. The t-test was then used to prove the significance of

the result and difference between the two groups. When using the data for well A1 and well B1, the t-test performed using the raw data showed that the P-value of the statistics is 0.0498. This proves that the experiment conducted was a valid experiment. The mean of Group A was 6 and the mean of Group B was 3, proving that there was a big difference in the number of zebrafish that survived in each group. The SEM was only 0.41 for Group A, which also shows that the experiment was a valid experiment because the major of the data points were close to the average and had little deviation. The statistics were based on the data that was recorded for the dependent variable, the number of fish alive. The independent variable was the concentration for alcohol, which was 0% for group A, and 0.06% alcohol for group B.

Discussion

This experiment was conducted to show the effect alcohol exposure has on the mortality rate and spinal development of embryos to understand the detrimental effects of a woman drinking while she is pregnant. Many human embryos are exposed to alcohol while developing, and it is important to find out what detrimental effect alcohol has on mortality rate and deformations (Center for disease control and prevention, 2015). Although not drinking while pregnant seems obvious to many women, one in ten pregnant women report drinking in the last thirty days (Center for disease control and prevention, 2015). This results in at least 10% of babies being at risk for any complications that can be caused by alcohol exposure while developing (Center for disease control and prevention, 2015). It is important to understand how alcohol is affecting a developing embryo, and whether or not a small amount of alcohol still affects the embryo.

The data from the experiment partially supported the hypothesis. The data clearly shows that the zebrafish exposed to alcohol had a higher mortality rate and physical deformities; however, both experimental groups experienced similar mortality rate, and the 0.06% alcohol-exposed embryos had a slightly higher mortality rate. The alcohol-exposed zebrafish embryos did have a lower hatching percentage. The embryos exposed to alcohol also hatched sooner than those not exposed to alcohol. These findings show that the alcohol caused an abnormal development of the embryos and an abnormal timeframe for growth. These factors may have led to poorly developed organs that failed, which then would have caused the higher

mortality rate for the alcohol-exposed embryos. The fish exposed to alcohol that hatched ended up having curved spines, which once again shows that alcohol caused the fish to develop improperly. The alcohol caused the bone structure to develop differently than normal.

The results from this experiment line up with the results from other experiments conducted in which zebrafish were exposed to alcohol. As referenced in the introduction, other experiments have been designed to test the effect of alcohol on zebrafish development. A study scientists completed showed that when zebrafish embryos are exposed to alcohol, they do in fact have abnormal motor neurons and muscle fibers and an abnormal response to touch (Sylvain, Brewster, Ali, 2010). The zebrafish embryos exposed to alcohol did not move when touched, but normal zebrafish embryos did (Sylvain, Brewster, Ali, 2010). This experiment also shows that alcohol exposure causes abnormal development of the zebrafish embryo. Knowing this, the data is even more reliable because it follows the general trend of data that other scientists have collected.

The results show that alcohol does in fact affect embryonic development. Because zebrafish are model organisms, the data collected using zebrafish can directly correlate to the results of a mother drinking alcohol while pregnant (Burke, 2016). If a woman consumes alcohol while pregnant, the baby will be exposed to the alcohol through the umbilical cord and therefore have a higher chance of dying, or having developmental and neurological problems, just as the zebrafish did. The data from this experiment shows that alcohol-exposed embryos are at risk for physical deformities, like the curved spine of the embryo. There is also an increased chance of

miscarriage or having a stillborn baby, like many of the zebrafish. These are all problems that have been found to be in alcohol exposure human embryos and these problems are called Fetal Alcohol Syndrome (FAS) (“What are fetal alcohol spectrum disorders”, 2019). The effects from this condition can include physical abnormalities, neurological problems and organ failures (“What are fetal alcohol spectrum disorders”, 2019).

In conclusion, it is a very poor decision to drink while pregnant and in doing so, many mother risk the chance of their baby dying or having severe problems.

While carrying out the experiment, a few uncontrolled variables were present and a few experimental errors occurred. For example, the number of zebrafish fish alive in each well on the first day of the experiment varied. Around ten eggs were placed in each well, and then the dead eggs were taken out. This resulted in 5-10 eggs left in each well. Exactly 28 eggs were counted in the four wells for the 0% alcohol concentration, whereas there were 30 eggs for the 0.06% and 0.3% concentration. In order to perform a more precise and scientifically significant experiment, the number of eggs in each well should have remained constant. Another experimental error occurred when three eggs were accidentally placed into the waste beaker. Sometime during the process of discarding waste solution and adding a new solution, three eggs were sucked into the micropipette and placed into the waste beaker. This was discovered when the well plate was taken out of the incubator at 72 hours post fertilization, and there were two fewer eggs in well B2 then there were supposed to be. This affected the experiment because it is unknown whether or not those eggs would have survived. Being in a classroom

setting with a limited amount of time and a limited amount of zebrafish eggs, caused the experimental procedure to only include four wells of each treatment. If the experiment was repeated in a different setting, more wells could be used to collect more accurate data. These experimental errors most likely are the reason why the 0.06% alcohol-exposed group had a lower mortality rate than the 0.3% alcohol-exposed group; however, the results still showed higher mortality rates and physical deformities for the alcohol-exposed embryos.

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