The Effect of Nicotine on Mortality Rate and How it is Possibly Linked to Miscarriages
Would You Smoke if it Cost a Life?

Savannah Arteaga, Maddie Nelson, & Morgan Skoluda
Waukesha North High School

Abstract

Nicotine is a harmful chemical that comes from a plant called tobacco. Nicotine has been found to be the cause of many health problems for people who use this chemical, a more fatal result of using of using nicotine is even death. In the experiment conducted, nicotine was added to four wells with 10 zebrafish embryos in each. The following amounts of nicotine were used (0.0, 0.05, 0.1, 0.2 mg/mL). This was done over the course of 72 hours (3 days). The experiment was based on the hypothesis that the more nicotine solution the zebrafish embryos were exposed to, the fewer the number of eggs would hatch. The dependent variable in the experiment was the mortality rate of the embryos. The independent variable in the experiment was the four different amounts of nicotine solution. After collecting the data over the 72 hours, a chi-square analysis was done to prove the hypothesis correct. The ending results show that the higher the nicotine concentration, the less amount of eggs hatched. This means that the hypothesis was not by chance, and the nicotine solutions did have an effect on the embryos. This experiment was done to make people, pregnant women in particular, aware of the dangers of smoking.

Introduction

Zebrafish are known to be a great model for experimenting on, from uwm.edu, "...an excellent model for teaching high school students the basic principles of vertebrate

development. The zebrafish embryo exhibits several characteristics that make them attractive models". Some of the reasons behind zebrafish being good models are that they develop outside the mother, are produced in large numbers, exhibit occurring development at the same time within a group, are mostly clear, and develop rapidly.

Zebrafish don't take nearly as long to develop as other test subjects do, "They grow at an extremely fast rate, developing as much in a day as a human embryo develops in one month." This is good for the reason that you can experiment on them and get efficient data without it taking months or even years to do. Another good thing about using zebrafish for test subjects is that they share a high percentage of the same genes as humans, this means they have a similar genetic structure. They are known to have about 70 percent of the same genes as humans. According to yourgenome.org, "As a vertebrate, the zebrafish has the same major organs and tissues as humans. Their muscles, blood, kidney, and eyes share many features with human systems." If you were to do some sort of experiment using zebrafish this shows that the results would be very similar compared to the results that would come from a human test subject.

The purpose of this experiment was to observe the effects of increasing amounts of nicotine on developing embryos and to draw a correlation to human fetal development. Approximately 12-22% of the population, or 1 million women in the United States smoke while pregnant. Smoking while pregnant can cause, premature birth, low birth weight, cognitive defects and behavioral problems in babies and toddlers. This experiment was conducted on zebrafish embryos to show people the possible effects of nicotine on the human fetus, and show how it has been linked to miscarriages. It is important to show how nicotine can have a negative impact on pregnant mothers and their babies, if we show the effects now, pregnant women might stop smoking in the future. According to the Faculty of Dentistry Center of Studies for Periodontology, "The risk of miscarriage increased with the amount smoked (1% increase in relative risk per cigarette smoked per day)."

Secondhand smoke comes from the end of a cigarette, cigar, pipe, and the smoke that is exhaled by a smoker. According to medlineplus.org, "It contains more than 7,000 chemicals. Hundreds of those chemicals are toxic and about 70 can cause cancer." If all of these chemicals are being inhaled by a pregnant woman, it is almost guaranteed to

affect a newborn baby and even the fetus that's developing in the womb. Adding on to this a little bit more, "Secondhand smoke exposure during pregnancy increased the risk of miscarriage by 11%." Even if this doesn't seem like that big of a number, it can make a big difference and there can always be other factors that could increase this percentage.

Overall secondhand smoking is neither good for the baby fetus and its health, or the pregnant mother herself. The correlation between the zebrafish embryos and the nicotine solution is to see if when the substance was added to the embryos, it would have an effect on fetal development of the eggs. If the zebrafish embryos are exposed to nicotine then the number of eggs hatched will decrease, this is because nicotine has been linked to increasing the risk of miscarriages.

Materials & Methods

The materials included in this experiment were a 100 mL beaker, multi-well tray with 12 holes and a cover, Sharpie, 3 strips of blue tape, iPad, student data sheet, Instant Ocean/Embryo Media Solution, large bore transfer pipette, minimum bore 1.5 mm transfer pipette, 28.5 °C incubator, dissecting microscope, Nicotine solutions (0.0, 0.05, 0.1, 0.2 mg/mL), and last is the 40 zebrafish embryos.
Gloves should have also been worn so that there was no contact with the nicotine solution and skin.

First, the multi-well tray (fig. 1) was labeled with the nicotine solutions. The first row was labeled with the amount of nicotine solution in the order (0.0, 0.05, 0.1, 0.2 mg/mL). In the first row which contained four wells, 10 zebrafish embryos were placed in each, with a total of 40 embryos. The 40 were chosen by the appearance of their embryos. If the embryos were translucent, then they were alive and healthy. If they were white and cloudy, then they were most likely dead and not chosen. Any residual transfer liquid was removed using a fine bore pipette and replaced with the corresponding nicotine solution. Over the four days of the experiment, everyday the zebrafish were looked at with the dissecting microscope, they were then counted and recorded with the student data

sheet. There were also notes and features that were written down about the zebrafish, the main focus that was observed throughout the experiment was the number of eggs that didn't hatch or died before hatching at all. After each well was observed, pictures were taken with an iPad. This was so that you could see the differences and changes over the four days. If there ended up being dead ones, they were removed with the fine bore transfer pipette and put into the 100 mL beaker. Every day the nicotine solutions were replaced with new solutions, with 1mL of each (0.0, 0.05, 0.1, 0.2 mg/mL). The first well (0.0 mg/mL), was replaced with the Instant Ocean/Embryo Media Solution because this solution contained no nicotine and was the control group, this was done with the large bore 1.5 mm transfer pipette. The solution and if any dead embryos were in the well, they were extracted and put into the beaker and discarded. When done with observing, recording, and taking pictures of the embryos, they were placed in a 28.5 °C incubator. A chi-square analysis was completed on the data to ensure statistical significance.

[Costar® 12-well Clear TC-treated Multiple Well Plates, Individually Wrapped, Sterile]. (n.d.).
   Retrieved from https://ecatalog.corning.com/life-sciences/b2c/US/en/Microplates/
   Assay-Microplates/96-Well-Microplates/Costar%C2%AE-Multiple-Well-Cell-Culture-Plates/p/3513

Figure 1: Multi-Well Plate
This is the multi-well plate used to view the zebrafish embryos during the experiment

Results

The purpose behind this experiment was to make people aware of the dangers of pregnant women smoking. It was conducted to prove the hypothesis that the number of eggs hatched would decrease due to nicotine. This experiment was set up with the different nicotine solutions (0.0, 0.05, 0.1, 0.2 mg/mL) to correlate how the more nicotine inhaled and put into the body while pregnant, would increase the risk of miscarriages each time nicotine was inhaled.

   The dependent variable in this experiment was the mortality rate of zebrafish embryos. The independent variable is the different nicotine solutions. The control group in the experiment was 0.0 mg/mL of nicotine. Some of the controlled variables included were the same size well, the same amount of each solution, the same incubator temperature, and each well had the same amount of embryos in it. The dependent and independent variables relate to each other because as the concentration of the nicotine solution increases, then the number of embryos hatched will decrease (table 1 and figure 2). The chi-square for the live zebrafish embryos was 6.32, and the chi-square for the dead zebrafish embryos was 13.16. Both of these calculated together would be 19.48, this was found by adding up the live chi-square and the dead chi-square. The degree of freedom that was used on the distribution table was 3, and the probability used was 0.05. Our results were found by going to the degree of freedom (3), then finding the totaled number of chi-squares (19.48). If the totaled chi-square was greater than the critical value of 7.82, it would mean that the null hypothesis was rejected. This means the results show that the outcomes of this experiment were not by chance and that the end nicotine did have an effect on the embryos. The purpose of this experiment was to see if the nicotine solution, would have a negative effect on the zebrafish embryos used, which it did.

Table 1: Number of Hatched & Living Embryos Post Fertilization

This data table is showing the effects nicotine has on the number of zebrafish embryos hatched, over the course of a 72 hour span. The data shows that the higher the nicotine solution the embryos were exposed to, the less likely it would be for them to hatch.

Treatment	# of starting fish	24 hours post fertilization		48 hours post fertilization		72 hours post fertilization	
		# Hatched	# Live	# Hatched	# Live	# Hatched	# Live
0.0 mg/mL	10	0	10	7	10	10	10
0.05 mg/mL	10	0	10	9	10	10	10
0.1 mg/mL	10	0	10	3	8	4	3
0.2 mg/mL	10	0	10	2	9	6	4

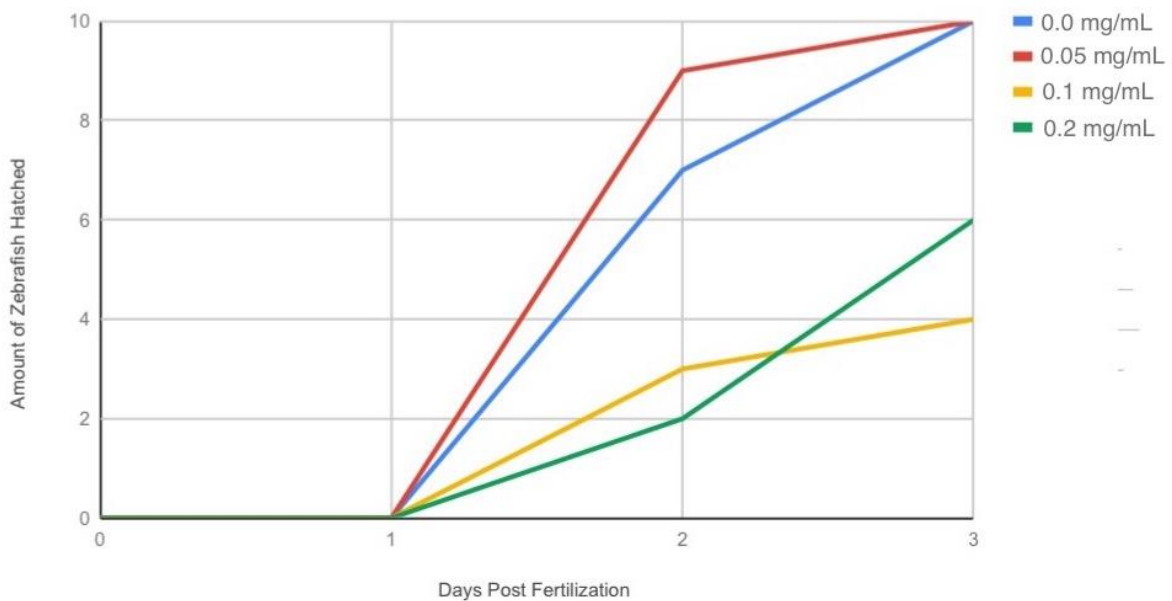Amount of Zebrafish Exposed to Nicotine Hatched Overtime

Figure 2: Amount of Zebrafish Exposed to Nicotine Hatched Overtime
This line graph shows the relationship between how many zebrafish hatched, over the span of the 3 days post fertilization. The data is showing that the higher the nicotine solution the embryos were exposed to, the less number of embryos hatched.

Discussion

When women smoke while pregnant, it can increase the risk of having a miscarriage. The ending results in this experiment show that smoking when a woman is pregnant, can cause many different risks for a baby fetus and the mother who is carrying it. Miscarriages are a major one of them. The exposure of zebrafish embryos to nicotine was a way of showing what can happen to a baby fetus. The experiment done was to see if the substance used, nicotine, would have an effect on the embryos. The research showed that nicotine is a harmful chemical that can increase the risk of a miscarriage by at least 11%.

The outcome of the results show that the hypothesis in this experiment was not by chance, and that there was an increase in mortality rate during the 72 hours post fertilization. The outcomes of the results show that the original hypothesis in the experiment was accepted, and this is why. In figure 2, depending on the amount of substance used, the line on the figure starts to increase. But, the embryos hatched will only rise to a certain number depending on the amount of nicotine that they're exposed to. This is how the research done and the outcome of the results relate to each other.

The test subjects in this experiment were the zebrafish embryos. The embryos were used in this experiment because of the many similarities between zebrafish and humans. This means that the results of the experiment would most likely be the same if done on a baby fetus, instead of zebrafish embryos.

During this experiment, some limitations were the small amounts of sample sizes, only using the four solutions of nicotine, the short amount of time the experiment was done in, the limited amount of observations written down about the embryos, and that the experiment was only done once.

Something things that could be done differently to improve this experiment are a larger number of zebrafish embryos, higher concentrations of nicotine solution, a longer time period to observe the embryos, write down more information on the observations of the embryos each day, and to repeat this experiment multiple times,.

A source of error that occurred in the experiment was transferring the dead embryos from the wells, to a separate beaker using the fine bore pipette. This was for the reason that the embryos were small, so it was hard to extract one without extracting the rest.

Something that could be further researched and collected data on, is how long it would take for the zebrafish embryos to all hatch completely. Also, using a larger number of the test subject (zebrafish embryos) and more amounts of nicotine solution.

Even though the data collected didn't exactly correspond with the ending amount of eggs hatched, meaning less number of eggs hatched for the higher amounts of concentration, it still proved to be a successful experiment. This experiment was done to show how nicotine can affect pregnant women and the baby fetus in negative ways.

Referenced & Literature Cited

Arffin, Farha, et al. "Environmental Tobacco Smoke and Stress as Risk Factors for Miscarriage and Preterm Births." *Archives of Gynecology and Obstetrics*, vol. 286, no. 5, Nov. 2012, pp. 1187–91. *Springer Link*, doi:10.1007/s00404-012-2417-0.

Petzold, Andrew M., et al. "Nicotine Response Genetics in the Zebrafish." *Proceedings of the National Academy of Sciences*, vol. 106, no. 44, Nov. 2009, pp. 18662–67. *www.pnas.org*, doi:10.1073/pnas.0908247106.

Pineles, Beth L., et al. "Systematic Review and Meta-Analysis of Miscarriage and Maternal Exposure to Tobacco Smoke During Pregnancy." *American Journal of Epidemiology*, vol. 179, no. 7, Apr. 2014, pp. 807–23. *PubMed Central*, doi:10.1093/aje/kwt334.

Secondhand Smoke. https://medlineplus.gov/secondhandsmoke.html. Accessed 26 Nov. 2019.

Spindel, E. R., & McEvoy, C. T. (2016). The role of nicotine in the effects of maternal smoking during pregnancy on lung development and childhood respiratory disease. Implications for dangers of e-cigarettes. *American Journal of Respiratory and Critical Care Medicine*, *193*(5), 486–494. https://doi.org/10.1164/rccm.201510-2013PP

Substance use during pregnancy | cdc. (2019, July 24). Retrieved November 11, 2019, from https://www.cdc.gov/reproductivehealth/maternalinfanthealth/substance-abuse/substance-abuse-during-pregnancy.htm

"Why Use the Zebrafish in Research?" *Yourgenome*, /facts/why-use-the-zebrafish-in-research. Accessed 20 Nov. 2019.

Wickström, R. "Effects of Nicotine During Pregnancy: Human and Experimental Evidence." *Current Neuropharmacology*, vol. 5, no. 3, Sept. 2007, pp. 213–22. *PubMed Central*, doi:10.2174/157015907781695955.