How Developing Zebrafish are Affected by Nicotine

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Abstract

Twelve to twenty percent of women smoke while pregnant, putting their children at risk for underdevelopment. 1000 babies in the US die each year due to their mothers smoke while they are pregnant. The chemical that causes this is called Nicotine. Nicotine is a substance found in most tobacco products. The zebrafish embryos are used as an example to simulate what happens to fetuses when they absorb nicotine. The embryos were placed in fluids that contain different amounts of nicotine. They were in the fluid for 24 hours before the fluid was replaced and new fluid was added in and data was recorded. The experiment lasted for 96 hours. During that time, the embryos in a higher concentration seemed to die or develop slower in comparison to the control. All of this data relates to the amount of nicotine that can harm a developing fetus.

Introduction

According to the CDC, 20.8% of teens use an e-cigarette, 8.1% use cigarettes, 7.6% use cigars, 5.9% use smokeless tobacco, 4.1% use hookah, and 1.1% use pipe tobacco all of these products contain nicotine. This addiction at an early age can be harmful because addiction can be hard to stop when someone is pregnant. ”In the US, over 1,000 children die because their mothers smoked while pregnant” (American Pregnancy Association). Zebrafish embryos were used to mimic what would happen to a developing fetus who exposed to nicotine or secondhand smoke. According to Johnson (2018), “If you are regularly exposed to secondhand smoke while pregnant, you will have an increased chance of having a stillbirth, a low birth weight baby, a baby with birth defects, and other complications of pregnancy.” Exposure to nicotine can damage embryos, “Any zebrafish embryo exposed to nicotine will be paralyzed. This is an indication that nicotine has affected the motoneuron and/or muscle development”
(Svoboda). The experimental hypothesis tested was, **if increasing amounts of nicotine are added then the embryos will develop slower than the control that does not develop fully in comparison to the control because when nicotine is present zebrafish become paralyzed and immobile.**

Materials and Methods

The procedure sheet for this lab was used as a guide. The four wells were labeled with the group's name, hour and the fluids with blue tape and a black sharpie and 10 embryos were placed in each well. Residual transfer fluid was removed and the wells each had 1 mL of their proper fluid placed in the wells through a pipette. When placing the fluid into the wells, gloves were worn when placing the nicotine into the wells. The fluid was replaced by using a pipette and removing any dead embryos as well as the old fluid. One pipette was used in the order of the lowest nicotine concentrate to the highest. Then, the new fluid was placed into each well. The embryos were examined through a microscope. Any dead embryos and fluid were removed and replaced with 1 mL of new fluid. The embryo shells and fluid were removed and replaced. Survival rate and hatching rate were measured. Hatching rate was measured by the embryo completely out of its shell. The survival rate was determined by the embryo turning a black or dark color and showing no signs of development. The hatching rate and development rate were recorded on a table (Table 1). A chi-square analysis was performed to ensure statistical significance.

Quantity	Item
1	Blue Tape
1	Black Sharpie
1	Well Plate
1	Beaker of fluid with 0.00, 0.05, 0.10 and 0.20 mg/mL of nicotine
1	An iPad was used to take pictures of the embryos progress
1 (unless more were needed)	Pipettes
1	Microscope
1	Glass Beaker 400 mL The glass beaker was used for disposal of used liquid and embryo shells
1	Incubator 28.5 °C

Results

The experiment was done to simulate what would happen if an embryo was exposed to a large amount of nicotine. This experiment measured the number of embryos that hatched (Figure 1) in different concentrations (0.00 mg/mL, 0.05 mg/mL, 0.10 mg/mL, 0.20 mg/mL) of nicotine solutions in comparison to the control. The independent variable is the different concentrations of nicotine and the dependent variable is the hatching rate of the embryos. Our Control was 0.0 mg/mL and the constants in the experiment were the temperature of incubator, amount of embryos in each well, and the size and the amount of fluid in each well. To test for independence of nicotine solution and hatching rate 1 mL of the respective fluid placed in each well. All of the embryos were placed in an incubator at 28.5 °C. As the concentration of nicotine increases, more embryos die or are paralyzed (Figure 2). The embryos in the control were very active and on the last day to test their reflexes, they were chased with a pipette the control fish were very responsive and moved away quickly. The higher the concentration the less responsive the embryos were to the pipette. A chi square analysis was used to test for independence of the nicotine solution and hatch rate. The chi-square value was calculated and the total came out to be 40. Using a degree of freedom of 3 and the critical value being of 7.82 the null hypothesis was rejected because the nicotine concentrations did have an effect

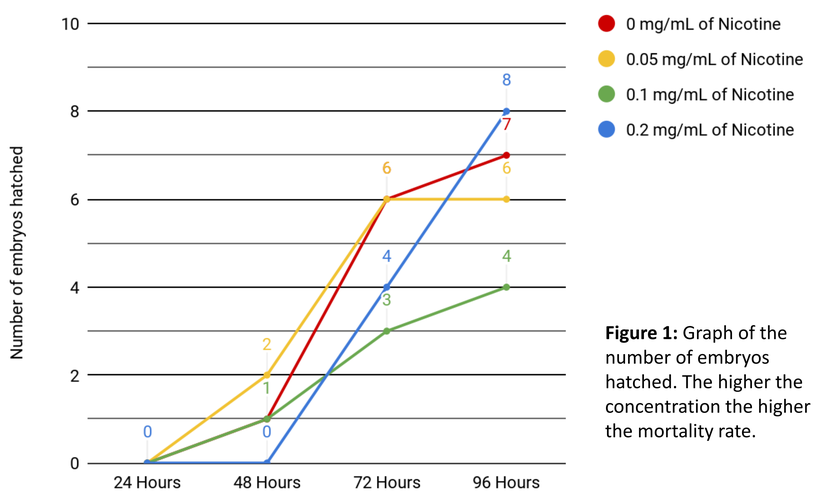

Figure 1: Graph of the number of embryos hatched. The higher the concentration the higher the mortality rate.

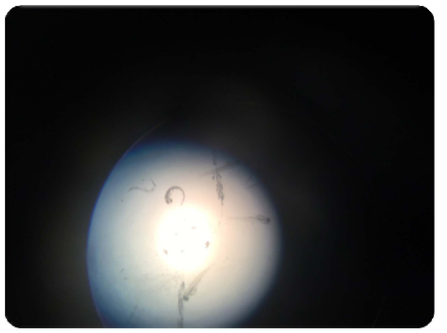

Figure 3: Embryos exposed to 0.00 mg/mL of Nicotine (72 hours)
The embryos have hatched and are acting normal.

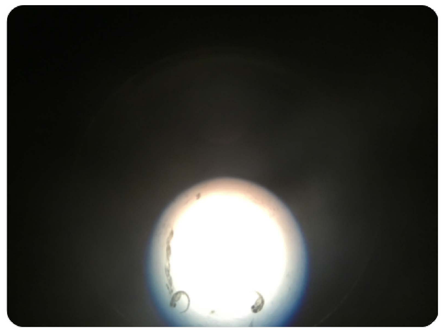

Figure 4: Embryos exposed to 0.20 mg/mL of Nicotine (72 hours)
The embryos have hatched but they appear to be curved

Discussion

The research of the effects of nicotine on fetuses and zebrafish embryos stated the initial hypothesis was, if increasing amounts of nicotine are added then the embryos will develop slower than the control that do not develop fully in comparison to the control because when nicotine is present zebrafish become paralyzed and immobile. The hypothesis morphed into, if increasing amounts of nicotine are added then the embryos will become less active than the control in comparison to the control because when nicotine is present zebrafish become paralyzed and immobile. This was found to be true because any exposure to nicotine means the embryo or fetus is more likely to die or have severe development issues. Increasing amounts of nicotine have shown increasing development issues in the embryos. The original hypothesis was found to be untrue because the zebrafish in the 0.05 mg/mL liquid developed just as much as the control but did not move. A larger sample size would be needed if doing this experiment again, as well as a scheduled time to take pictures for example after the fluid was removed and replaced. Errors that could have occurred are some of the lower concentrate embryos would have still been alive bet acted or appeared dead. The embryo wells were set up in the order of 0.00 mg/mL, 0.10 mg/mL, 0.05 mg/mL, 0.20 mg/mL the pipette may have still had some fluid from the 0.10 mg/mL well and contaminated the 0.05 mg/mL embryo well. To prevent this error from occurring, the wells should have been set up in the order of 0.00 mg/mL, 0.05 mg/mL, 0.10 mg/mL, 0.20 mg/mL when using one pipette or one pipette should be used for each well being tested. Nicotine can harm growing embryos and fetuses with even a very low concentration of nicotine.

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