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The Effects of Nicotine on the Development and Mortality Rates of Zebrafish Embryos

Abstract

More than one billion people consume something on a daily basis that is as addictive as heroin, nicotine. Although some studies suggest that it may increase memory, most studies suggest that it is a major risk factor to both the consumer and babies. In this experiment, 21 zebrafish embryos were exposed to 100 μM nicotine levels. All of the embryos were exposed to this concentration of nicotine. Our first set of embryos were exposed for 24 hours, our second set for 48 hours, and our third set for 96 hours. The mortality rates and deformities were both observed at each time period. Each day the solution was changed and the dead embryos were removed. The embryos are used to represent the baby as a fetus. It was found that nicotine increases the amounts of deaths and deformities, but how long they are exposed does not affect it significantly. Although many people know the side effects of nicotine, they still choose to consume it. Hopefully once expecting mothers see the full effects of nicotine on their children, they will quit smoking.

Introduction

The effects of nicotine on human embryos has been an ongoing study between scientists and researchers for several years. In contribution to these studies, another lab was run on the effects nicotine has on Zebrafish. Zebrafish were used for multiple reasons. Some being that the cost of them are relatively inexpensive, they have very similar embryos to a humans, and they have fairly transparent embryos (Dooley, 2000). Because of these reasons, zebrafish were the most logical animal to use in lab when studying the effects of nicotine on prenatal development of the embryo.

Nicotine affects both the consumer and the baby they are carrying in multiple negative ways. Nicotine will increase the babies heart rate, the chance of miscarriage or stillbirth, premature birth, birth defects, and sudden infant death syndrome (Parker and Connaughton, 2007). As for the carrier, it can lower the amount of oxygen available to her and the baby (Zhao, 2014). Not only will the nicotine affect the baby as a fetus, it will also affect the baby short and long term after birth. Short term due to the fact that the nicotine will stay in the mother's body during the time period where she would be breastfeeding which increases how long the baby is exposed to nicotine (Batra, 2004). It also affects the baby long term because coming from a mother who smoked during pregnancy, the child is more likely to have an addiction to nicotine (Felman, 2018) .

The question that this hypothesis composes is if the time exposed to nicotine will affect the mortality rates and birth defects? The hypothesis states that the longer the embryos are exposed to the same concentration of nicotine, then more fish will die or have deformities in response because nicotine causes birth defects and mortality. It is predicted that the same results that occur with zebrafish will happen to humans.

Materials and Methods

Materials:

- 21 zebrafish embryos
- Sanitized plate with wells (3x4)
- Tape (for labeling)
- Sharpie (for labeling)
- Instant Ocean/Embryo Media Solution (for controlled)
- Nicotine Solutions (.05 mg/mL, .1 mg/mL, .2 mg/mL)
- Dissecting and compound microscope
- 28.5 degree celsius incubator
- 1 mL disposable pipettes
- Waste container
- Depression slide with cover slip
- Methane blue (to prevent bacteria growth)

Procedure

Day 1-

1.) Obtain rinsed embryos from the teacher.

2.) Label the plate with a name and class hour. Label the nicotine concentration of each well using a Sharpie.

3.) Fill the one well of the plate with 1mL of Instant Ocean/Embryo Media solution using the disposable pipette. Fill the remaining wells with the appropriate ethanol stock solutions. Divide the embryos so there are approximately 10 embryos in each well. Label the plate on the student data sheet.

4.) Record exact numbers of live embryos on student data sheet. Discard dead embryos

5.) Observe the embryos under the dissecting microscope. Record observations on student data sheet.

6.) Place each plate in the 28.5℃ incubator overnight.

Day 2-

1.) Remove plate from incubator.

2.) Remove dead embryos from plate using the disposable pipette. Squirt dead embryos into waste beaker.

3.) Remove plate from incubator. Remove dead embryos from plate using the disposable pipette. Squirt dead embryos into waste beaker. Be careful to only remove dead embryos.

4.)Count remaining embryos, hatched fish, and record in data table.

5.)Remove caffeine solutions from each well of the plate.

6.)Replace the caffeine solutions with the appropriate fresh caffeine solution using a clean pipette each time.

7.)Place plate under dissecting microscope and record observations on student data sheet. Note/describe any developmental abnormalities and developmental markers. Repeat for all caffeine concentrations.

8.)Remove 1-2 embryos and place on the depression slide with cover slip. Observe the embryo using the compressed microscope. Record observations on student data sheet repeat for all caffeine concentrations.

9.)Return the embryos to the well in the plate.
Return the plate to the 28.5 degree Celsius incubator.

Day 3-

1.) Repeat Day 2 work and observations. Record all data.
If one does not wish to further the experiment, then place all embryos and fish in waste container. The teacher will properly dispose of the organisms.

Day 4-

1.) Repeat Day 2 work and observations. Record all data.

Day 5-

1.)Repeat Day 2 work and observations. Record all data. Place embryos and fish in waste container. The teacher will properly dispose of the organisms.
This procedure was created by SEPA - UW-Milwaukee

Data:

Data Table One

Embryos alive in instant ocean, 24 hours of exposure to 100 μM, 48 hours of exposure to 100 μM, and 96 hours of exposure to 100 μM.

Number of Living Embryos

Treatment	# of starting embryos	# of embryos alive 24 hpf	# of embryos alive 48 hpf	# of embryos alive 96 hpf
Instant Ocean Solution	28	26	23	19
100 μM Nicotine Solution	28	24	21	17

Figure One- Number of surviving embryos in 100 μM after 24 hpf, 48 hpf, and 96 hpf.

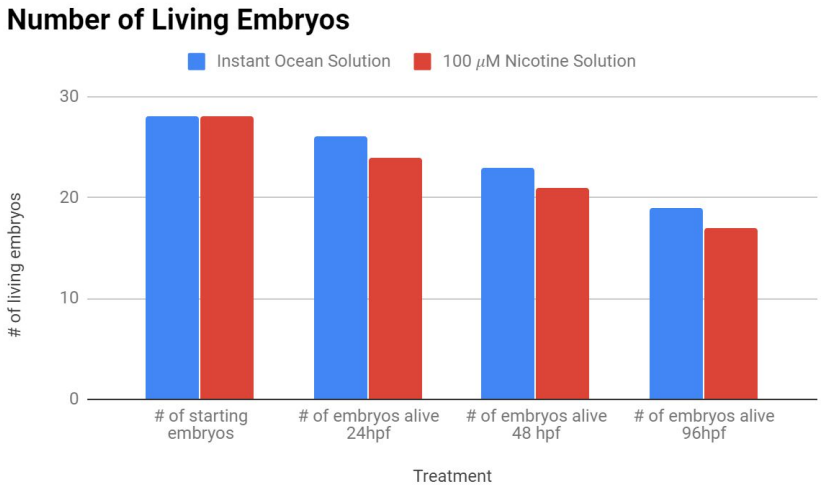

Figure Two- This image shows two zebrafish from the controlled group. This is the normal development of an embryo.

Figure Three- This image was taken from the zebrafish after 48 hpf in 100 μM. It shows defects like curved spines and enlarged abdomens.

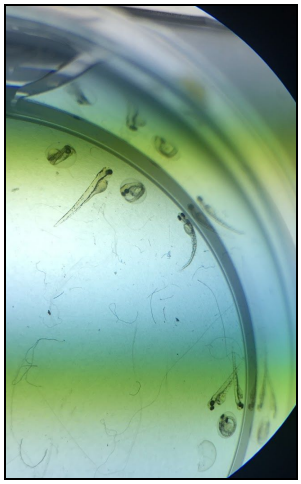

Figure Four- This image shows the zebrafish 48 hpf as well as premature development. This image also shows curved spines and enlarged abdomens as well.

Data Table Two:

Significance of Data in 100 μM Nicotine Concentration vs. Controlled

T test results which compares the controlled to each row of embryos.

Data Table Two		
Survival Rate		
Comparison	**P-value**	**Significance**
Controlled vs. 96 hours of exposure to 100 μM	0.6754	Not Statistically Significant
Controlled vs. 48 hours of exposure to 100 μM	0.3903	Not Statistically Significant

Controlled vs. 24 hours of exposure to 100 μM	0.0941	Not Quite Significant

Results

This experiment used three rows of embryos filled with 100 μM nicotine levels and one row as the controlled variable filled with instant ocean. The independent variable was how long the embryos were exposed in this concentration of nicotine. The dependent variable was the death rate of the embryos. The results showed very minimal impact on the zebrafish embryos and how long they were exposed to nicotine. The death rate increased very slightly the longer the embryos were exposed.

Discussion

The proposed question in this experiment was if the time exposed to nicotine would affect the mortality rates of the zebrafish embryos. Seeing as the T tests results shows no significance, the data shows that there is no correlation to how long embryos are exposed to nicotine and their mortality rates. This data does not support the hypothesis that states the longer embryos are exposed to nicotine, the more mortalities and defects. While doing this experiment, one error found was our limited supplies and class time. If we would have had a bigger sample of embryos and more class time to work, we would have received more accurate data. Some embryos may have also been contaminated or sucked up due to the limited time we had. While observing throughout the days, it was found that those exposed to nicotine for a longer amount of time had more deformities and premature development such as curved spines and enlarged abdomens. Figures 2 and 3 represent these observations. In support, WebMD states that nicotine affects the development of a baby by increasing the risk of a baby being born prematurely (Johnson, 2018). Although the length of time exposed to nicotine was not significant if this experiment, other observations were found that nicotine exposure does affect the development and mortality of embryos.

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