The Biological Effects of Nicotine on Zebrafish Embryos

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Abstract

This research is important because it shows the dangers of nicotine and how they could possibly affect humans. Over 34 million people smoke cigarettes in the U.S. daily. The purpose of exposing zebrafish embryos to nicotine is to see the effects that it has on vertebrates without using humans as a test subject. For this experiment, the zebrafish embryos were put in four different nicotine concentration solutions for 72 hours so that the negative effects of the different amounts could be recorded over time. This was done to understand the developmental delays, deformities, and deaths caused by nicotine. The data showed that the embryos exposed to the highest concentration of nicotine suffered the worst effects compared to those exposed to none. One of the hatched embryos exposed to nicotine was smaller in size than those in the control, this demonstrates that human embryos exposed to nicotine in the womb tend to have a lower birth weight, which can result in an array of medical problems. There is an abundance of connections to be made between the side effects of nicotine on zebrafish and exposure to developing human embryos. These research experiment sheds light on this.

Introduction

Over 1,000 babies die annually in the U.S. due to the mother smoking during pregnancy (American Pregnancy Association, 2017). The main cause of these deaths is a chemical known as nicotine. Nicotine is a harmful drug found in cigarettes, vape juices, and juuls. It is a stimulant derived from tobacco plants grown in the Southeastern parts of the United States (Felman, 2018). Those who use nicotine are prone to lung cancer, popcorn lung, and other diseases the average person would not as commonly face. During pregnancy what the mother consumes, the baby does as well. Women who smoke during pregnancy are more likely to have a miscarriage and have an increased risk for other severe diseases. Just 1-2 cigarettes can cause blood vessels to tighten" resulting in poor blood flow and oxygen to the fetus". The drug passes from the mom to the baby because the chemicals in the mother's bloodstream go to the baby, partially limiting the baby's only source of food and oxygen (Felman, 2018). A shortage of oxygen can cause the baby to be born prematurely or have a low birth weight that could result in developmental issues. "On average, a pack-a-day habit during pregnancy will shave about half-pound from the baby's weight" (Woolston, 2011). There are several other life long consequences put on the baby due to nicotine exposure "during the embryonic stages of life" including effects on the central nervous system and impairments on cardiorespiratory responses (The Physiological Society, 2018). Zebrafish embryos are often used in comparative embryonic studies because they have similar developmental structures to humans comparative vertebrates to humans (University of Oregon, 2015). Many women tend to switch to vaping during pregnancy instead of smoking, however, the effects are just as bad, as the baby still has a high chance of being delivered too early, having birth defects and potential trouble learning in the future (University of Oregon, 2015). The results of this experiment will provide a base for knowledge of the possible effects of nicotine on

humans and validate prior research. For this experiment and based on prior research, it is predicted that the embryos exposed to the highest concentrations of nicotine will be smaller in size with a 40% survival rate.

Materials and Methods

Materials

- 3 Bottles of Stock solutions of Nicotine (3 concentrations)
- 1 Beaker for dead embryos & liquid disposal
- 1 Sharpie
- 1 Bottle of Instant Ocean/Embryo Media Solution
- 2-3 Transfer pipette
- 1 3x4 Multi-well plate
- 1 28.5*C incubator
- 1 Glass Slide
- 1 Compound Microscope
- 1 Dissecting Microscope
- 1 Camera
- 20 Zebrafish embryos

Methods

On day one, rinsed zebrafish embryos were obtained to begin the experiment. The well plate was then labeled. The three nicotine concentrations and the control group were labeled as well next to the corresponding well plate. The top upper left well was filled with 1mL of the Instant Ocean solution using the wide pipette. The group then filled the experimental wells with the 3 concentrations of nicotine, Placing each amount in the matching previously labeled well. Using the narrow pipette, approximately 10 embryos were placed in each well. Making sure to take account of each live embryo by recording the exact number on the data sheet. By using a microscope, the group observed the embryos and recorded any observation onto the data sheet. To finish, the well plate is placed into a 28.5*C incubator overnight.

On the second, third, and fourth days, the well plate was removed from the incubator. Dead zebrafish embryos were removed from the plate by using the narrow pipette. The dead embryos were placed in the waste beaker. The remaining embryos were counted along with the hatched embryos and were recorded on the data table. The solution was removed from the wells. It is important to note that to settle the embryos at the bottom of the plate Fresh solution replaced the old solution via a clean pipette. Cross contamination was avoided by using a new, clean pipette for each solution. The plate was placed under the microscope and observations were recorded. The plate was returned to the incubator after usage.

On the fifth day, the well plate was removed from the incubator. Dead zebrafish embryos were removed from the plate by using the narrow pipette. The dead embryos were placed in the waste beaker. The remaining embryos were counted along with the hatched embryos and were recorded on the data table. The solution was removed from the wells. It is important to note that to settle the embryos at the bottom of the plate. Fresh solution replaced the old solution via a clean pipette. Cross contamination was avoided by using a new, clean pipette for each solution. The plate was placed under the microscope and observations were recorded. The plate was returned to the incubator after usage. All the data was recorded. The remaining embryos and fish were placed in the waste beaker. The organisms were then disposed of properly.

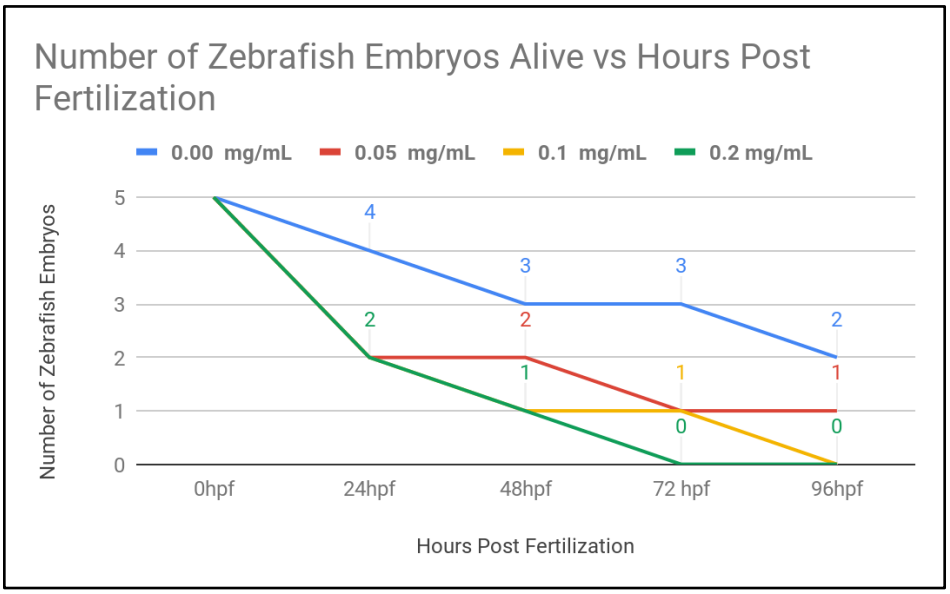
Safety Precautions

- No cross contamination
- Properly disposing of dead embryos

Results

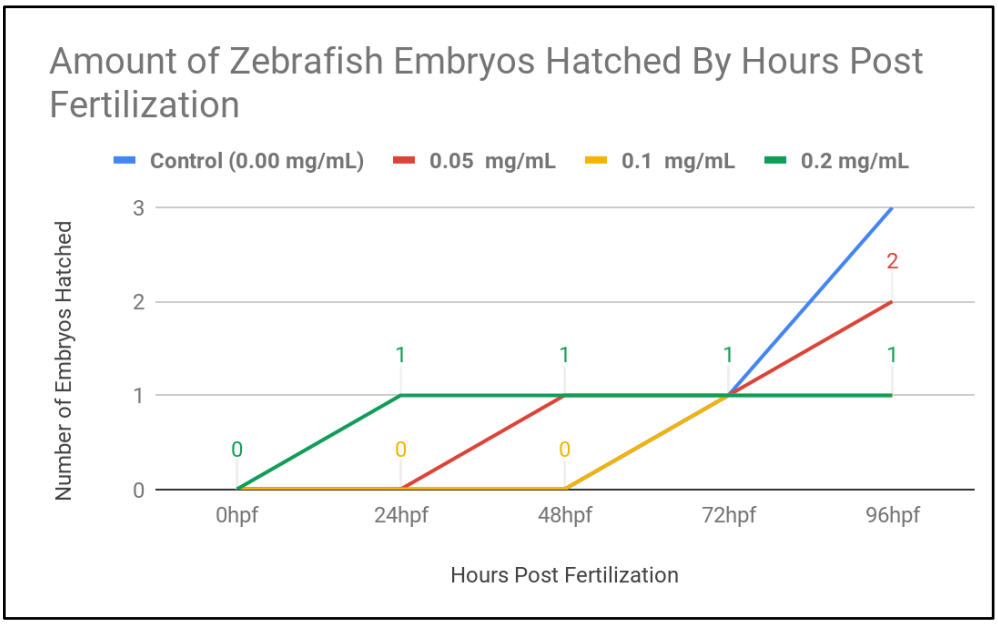
The objective of this experiment to prove how nicotine impacts vertebrate development. In this case, zebrafish embryos were used due to their similarity to humans in the fact they are both categorized as vertebrates. In this case, the deposited zebrafish embryos were placed into various concentrations of nicotine to represent what would happen if a mother were to smoke during her pregnancy. Using what was learned about zebrafish, it can then be expected to see some of the same results on humans. It was predicted that the embryos exposed to the highest concentrations of nicotine will be smaller in size with a 40% survival rate than those exposed to the lower concentrations of nicotine. Within this experiment the control variable was the instant ocean solution, every well had equal amounts of this base solution. It was made sure to not put any nicotine in the control well to keep data valid. The independent variable was how much nicotine was put into each well. For our dependent variable, five zebrafish embryos were obtained for each well to do this experiment on. On the final day, it was discovered that the embryos placed in the higher concentrations of nicotine had lower survival rates than the control group. It was also discovered that the embryos that were placed in higher concentrations of nicotine didn’t have as many eggs hatch as the embryos that were in lower concentrations of nicotine. One trend that was noticed in the experiment was that the eggs hatched faster in the highest concentration and in the control group, the eggs hatched the slowest. The eggs hatching faster in high concentrations of nicotine shows that nicotine can cause premature births.

Graph 1:



This graph displays how many embryos were alive vs hours post fertilization. It shows the slow depletion of the survivors throughout the days. The embryos died faster in the highest concentration of nicotine (0.2 mg/mL) while they died quite slower in the instant ocean (0.00 mg/mL).

Graph 2:



This graph displays how many embryos hatched by hours post fertilization. It shows that a total of seven eggs hatched out of the twenty eggs that w received. You can see that the embryos in the control (0.00 mg/mL) hatched the fastest.

Table 1:

Number of Embryos Alive vs Dead (Alive/Dead) at Different Concentrations of Nicotine over One Week

Time	hpf	Control Group (well #1) Alive / Dead	Experimental Group Concentration 0.05 mg/ml (well #2) Alive / Dead	Experimental Group Concentration 0.1 mg/ml (well #3) Alive / Dead	Experimental Group Concentration 0.2 mg/ml (well #4) Alive / Dead
Day 1	0 hpf	5/0	5/0	5/0	5/0
Day 2	24 hpf	4/1	2/3	2/3	2/3
Day 3	48 hpf	3/1	2/0	1/1	1/1
Day 4	72 hpf	3/0	1/1	1/0	0/1
Day 5	96 hpf	2/1	1/0	0/0	0/0

Table 2:

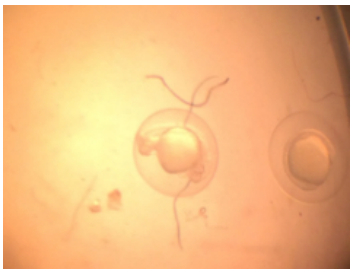
Number of Embryos Hatched vs Unhatched (H / U) at Different Concentrations of Nicotine over One Week

Time	hpf	Control Group (well #1) H / U	Experimental Group Concentration 0.05 mg/ml (well #2) H / U	Experimental Group Concentration 0.1 mg/ml (well #3) H / U	Experimental Group Concentration 0.2 mg/ml (well #4) H / U
Day 1	0 hpf	0/5	0/5	0/5	0/5
Day 2	24 hpf	0/4	0/2	0/2	1/2
Day 3	48 hpf	0/3	1/1	0/1	1/0
Day 4	72 hpf	1/2	1/0	1/0	0/0
Day 5	96 hpf	2/0	1/0	0/0	0/0

Picture 1:

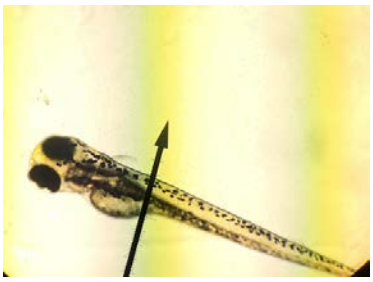
Day 1, All wells

Picture 2:



Day 2, control well.

Picture 3:



Day 4, control well

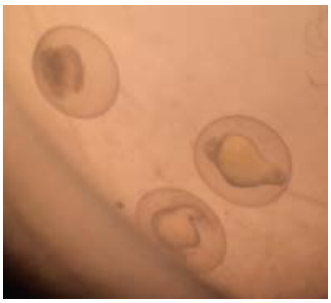
Picture 4:

Day 2, Experimental Well (#2)
Concentration of: 0.05m

Picture 5:

Day 4, Experimental Well (#2)
Concentration of: 0.05m

Picture 6:



Day 2,Experimental Well (#3)
Concentration of: 0.1 mg/ml

Picture 7:

Day 2, Experimental Well (#3)
Concentration of: 0.1 mg/ml

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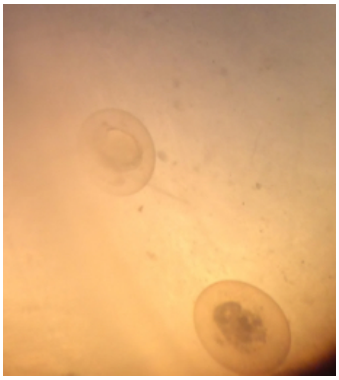
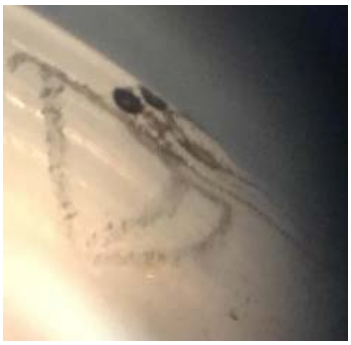
Picture 8:



Day 2, Experimental Well (#4)
Concentration of 0.2 mg/ml

Picture 9:



Day 4,Experimental Well (#4)
Concentration of 0.2 mg/ml

Discussion

The exposure of nicotine to zebrafish embryos can cause Sudden Death Syndrome and can double the chance of development deformities (Science Daily, 2018). Throughout the experiment, observations did not support the original hypothesis: embryos exposed to the highest concentrations of nicotine will be smaller in size with a 40% survival rate compared to those exposed to the lower concentrations of nicotine. It was seen that the embryos placed in higher concentrations had a higher mortality rate. Over time, more zebrafish embryos died in the experimental solutions than the control causing the survival rate to decrease. Although the zebrafish embryos in the experimental solutions died quicker than the control, the development of them was clearly being seen throughout the whole experiment. Through the observation of the zebrafish, there is a tendency shown that those exposed to higher nicotine concentrations will have an increased death rate. The well with the highest concentration (0.2 mg/ml) died at a faster rate than the lower concentrations and the control. On the other hand, the embryos in the highest concentration of nicotine hatched at a faster rate than those in the lower concentration and in the control. These early hatch rates correlate to human premature birth; premature births can cause defects, low birth weight, smaller size, and inferior physical health (2018, January 1.)

It was seen that the zebrafish embryos that were exposed to higher concentrations of nicotine exhibited a higher mortality rate than those compared to zebrafish embryos exposed to lower concentrations or embryos exposed to no concentration at all. Possible project errors including the incubator being set to a high temperature at the start of the experiment. An alarm that would let us know that the temperature was too high or too low in the incubator would have protected embryos from dying from other causes that were not being tested. Because the incubator was set to a higher temperature than there supposed to be instead of having 40 zebrafish embryos (10 in each well) there were 20 (5 in each well). Also, the experiment could’ve differed by using new

pipettes instead of reusing them. Although the pipettes were cleaned and sanitized they could have a trace of the chemical in the pipettes and therefore could have crossed contaminated. The experiment could have also varied by using new well plates, instead of old well plates were provided and they were cleaned and sanitized but there still could be old chemicals that were used for this experiment or other experiments.

Possible questions for further research could be regarding the change of mass of the zebrafish embryos when exposed to nicotine. If the zebrafish loses weight throughout the experiment, it could mean that they aren't getting enough nutrients and this could be fatal. Another research question could be, how the solutions could affect the zebrafish depending if the zebrafish came from the wild or captivity? If the zebrafish are in captivity there immune system may not be as strong if they were raised or found in the wild. If they were raised and found in the wild there immune systems might be stronger and can fight off toxins and dangerous chemicals easier.

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