The Effects of Nicotine on Embryonic Zebrafish Development

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Abstract:

The prevalence of the addictive drug, Nicotine, persists in society, taking on various forms from the traditional cigarette to youth-appealing e-cigarettes. With an adult consumption rate of 30.3%, an epidemic of addiction has taken over the United States ("Are there gender…"). As user side-effects are openly studied and observed, a conclusive finding of the effect of Nicotine on fetal development is alarmingly absent. In order to determine effects of the stimulant drug on fetuses, soon-to-hatch zebra fish eggs were placed in wells containing 2mL of 4 different nicotine solutions: the control (instant ocean), 0.05 mg/ml, 0.1 mg/ml, and 0.2 mg/ml. Throughout 2 days of observation, the fetal zebrafish within solutions containing higher concentrations of nicotine experienced increased rates of stunted growth, abnormal nervous systems, curved spines, and ultimately death. Zebrafish, having similar genetics and sharing the same major organs and tissues as humans, serve as valuable models of humans for scientific research ("Why Use the Zebrafish…"). Seeing as though nicotine negatively affects the fetal development of zebrafish, the human consumption of nicotine whilst pregnant will negatively affect human embryonic growth as well.

Purpose Statement/Objective: What is the effect of Nicotine on Zebrafish Development?

Background Research:

Nicotine, an addictive stimulant drug, is highly prevalent in society despite its numerous effects on the human body. The consumption of the substance results in rapid messages traveling between the brain and body, stimulating the nervous response of the release of hormones such as adrenaline ("What is nicotine?"). The side effects of nicotine vary from nausea and dizziness to fatal long-term effects such as aortic aneurysms and strokes. ("What is nicotine?") Despite the

fatal long-term ramifications, as of 2015, “16.7 percent of adult males and 13.6 percent of adult females smoked cigarettes” (“Are there gender…”), and therefore society’s adult population with a 30.3% consumption rate.

Since 2014, a surge of e-cigarette usage has surpassed cigarettes; therefore the primary consumption of nicotine in society can be attributed to e-cigarettes. The variety of sweet flavors found in e-cigarettes serves a primary role in the lower consumption of plain cigarettes. The flavor is considered the third most influential reason leading to adolescents’ experimental consumption of nicotine (Zare et al.). Adolescents’ consumption of nicotine is alarmingly prevalent; as of 2020, one in five high schoolers currently use e-cigarettes (Cullen et al.).

Previous to 2014, tobacco was the main source of nicotine intake. It can be consumed in many ways, the most common is smoking whether that be in a pipe, cigar, or cigarette. The endorphins and dopamine released, make the consumer feel false happiness that becomes highly addictive. 1.3 billion people worldwide consume nicotine leading to 8 million deaths yearly, from first and secondhand smoke (Tobacco).

Nicotine has many negative impacts on developing fetuses or babies in different ways, such as preterm birth, low birth weight, and birth defects in the mouth. Nicotine can damage a developing fetus' brain and lungs in many ways and can be severely harmful to babies. The most severe impact nicotine can have is death. A study done by the US CDC between 2007-2011 found that death risks rise by .07 for each cigarette smoked a day by a mother while pregnant (LaMotte 1). While the more nicotine one consumes, the more likely their baby is to experience unexpected death, even just one or two can have an impact on a baby's life. Research done by the American College of Obstetricians and Gynecologists also found a direct connection between the consumption of nicotine and SIDS (Sudden Infant Death Syndrome), which found that anywhere

between 23-34% of SIDS cases and 5-7% of pre-birth-related deaths can be connected to nicotine consumption before the birth of the child (LaMotte 2).

Hypothesis:

Null: An increase in Nicotine will have no effect on Zebrafish development.

Alternative: An increase in Nicotine will negatively affect a Zebrafish's development.

Materials:

- One 3x4 well plate
- Instant Ocean Solution
- 0.05 mg/ml nicotine solution
- 0.1 mg/ml nicotine solution
- 0.2 mg/ml nicotine solution
- Methylene Blue
- 96 Zebrafish Eggs
- Pipettes

Methods:

Day 1:

1. Using a pipette, each well in the first column (the control group) was filled with 2mL of Instant Ocean. See Figure 1
2. Using a pipette, each well in the second column was filled with 2mL of a 0.05 mg/ml nicotine solution. See Figure 1
3. Using a pipette, each well in the third column was filled with 2mL of a 0.1 mg/ml nicotine solution. See Figure 1
4. Using a pipette, each well in the fourth column was filled with 2mL of a 0.2 mg/ml nicotine solution. See Figure 1
5. A clean pipette was dipped into the methylene blue solution, the pipette was then dipped into a well. This step was repeated for each of the 12 wells.
6. Using a pipette, 8 zebrafish eggs were added to each of the 12 wells
7. The well plate was placed into an incubator set at 28.5 degrees Celsius and was left overnight.

Days 2-3:

8. The following day, each of the wells was examined using a dissecting microscope in order to observe the zebrafish. Quantitative data was recorded based on the examination.
    a. The Quantitative data recorded was the number of Fish that are alive (days 2-3), and number of Fish that hatch daily (days 2-3), and the physical development of fish (only on day 3)
9. From low to high concentration, the dead embryos were removed from each well.
10. The environmental factors were removed from each well using a pipette. To remove environmental factors the well was tilted, the embryos settled to one side and the liquid was removed from the top.
11. Steps 1-5 were repeated
12. The plate was placed into the incubator set at 28.5 degrees Celsius and left overnight
13. Steps 8-12 were repeated on day 3.

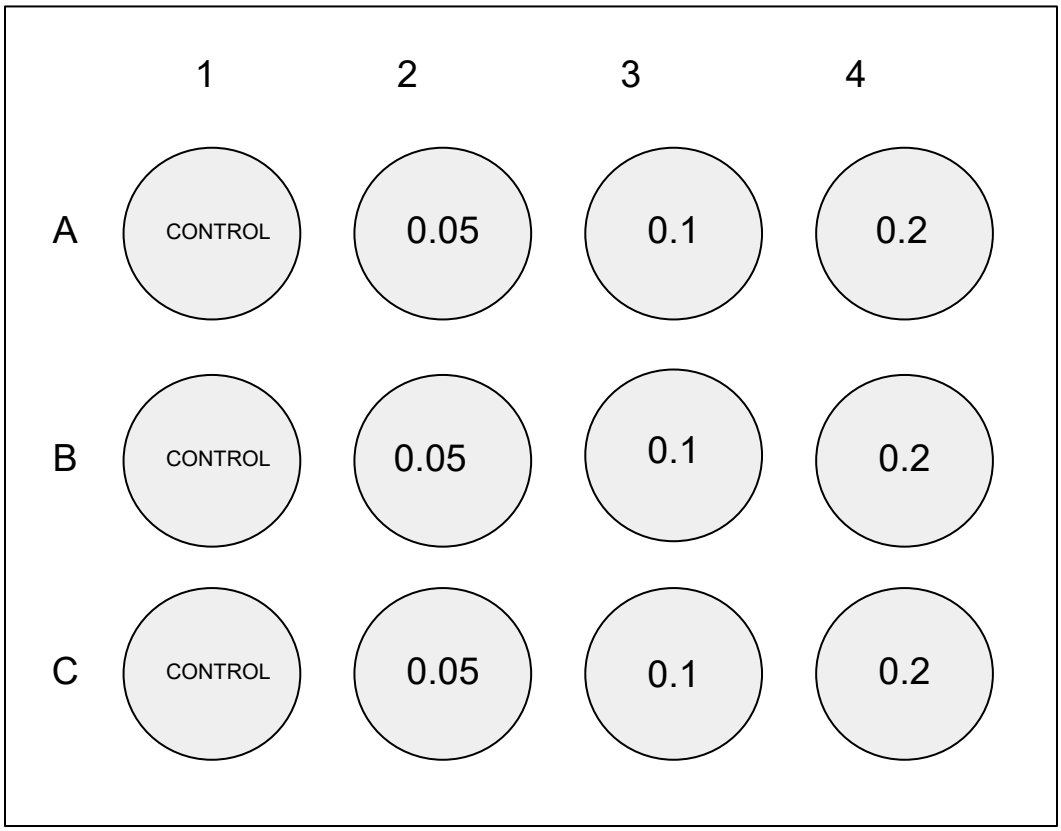


Fig. 1: Column 1 contains 2mL of Instant Ocean Solution, the control group. Column 2 contains 2 mL of an 0.05 mg/ml nicotine solution. Column 3 2mL of a 0.1 mg/ml nicotine solution. Colum 4 contains w mL of a 0.2 mg/ml nicotine solution.

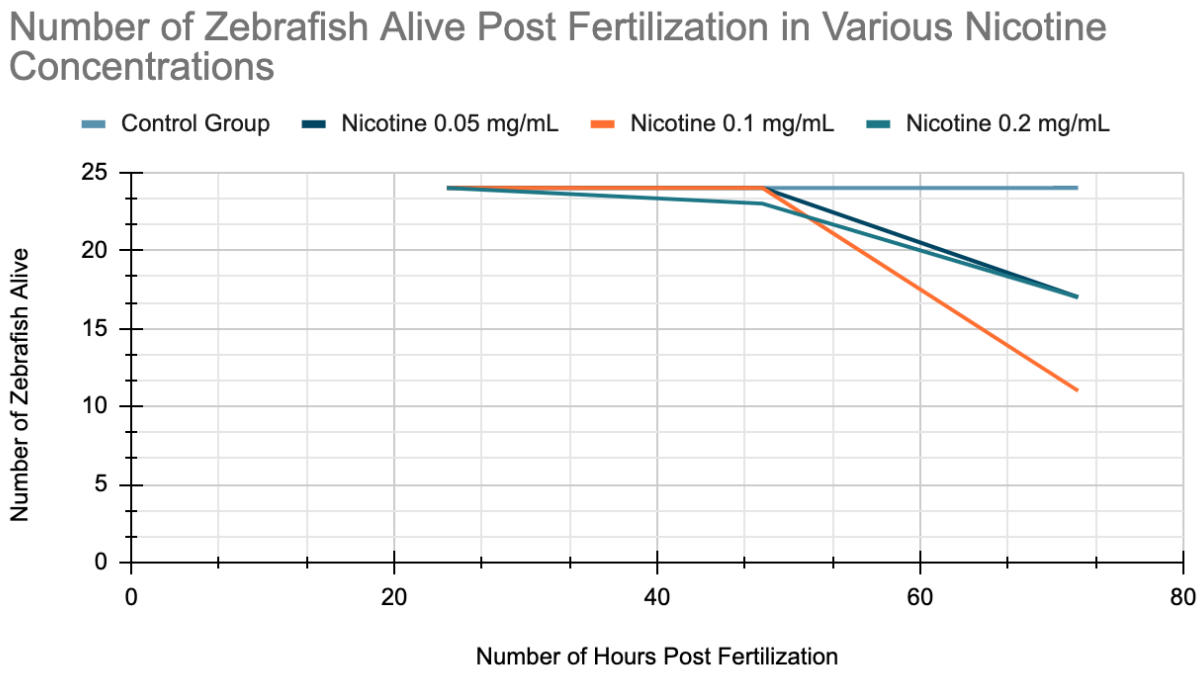
Table 1. 4 Solutions: Fish Hatched and Fish Alive Post Fertilization

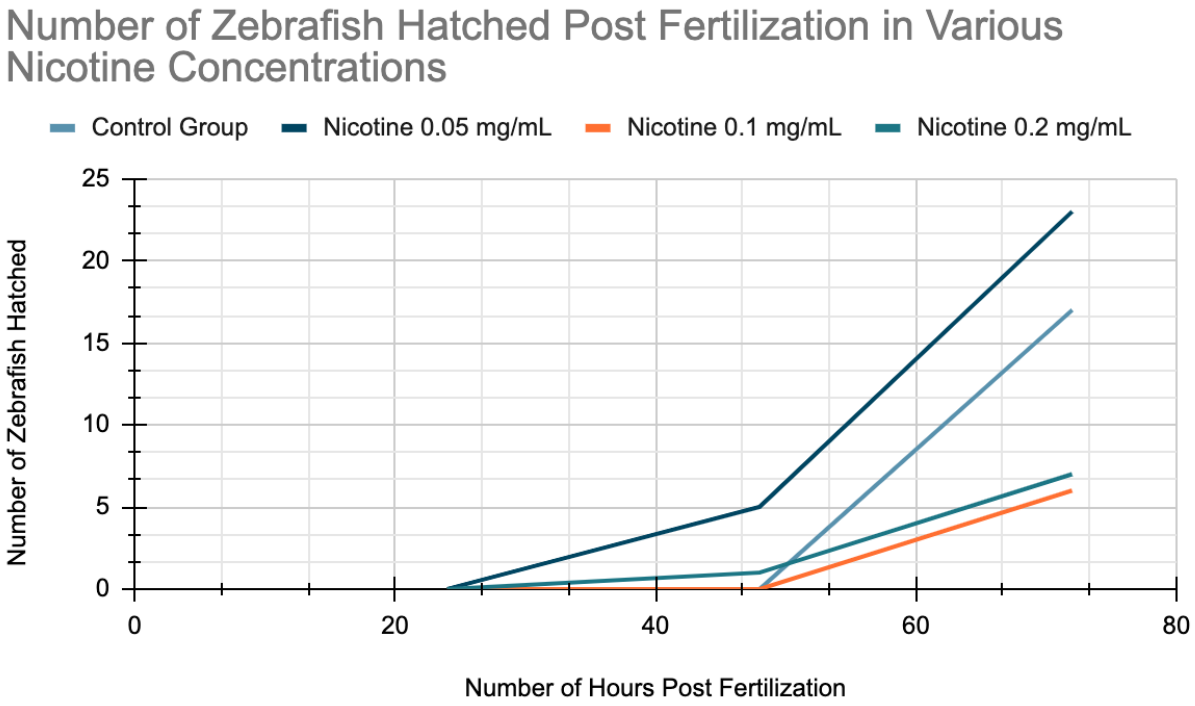
Treatment	Well #	# of starting fish	48 hours post fertilization		72 hours post fertilization	
			# hatched	# live	# hatched	# live
Control Instant Ocean	A1	8	0	8	6	8
	B1	8	0	8	5	8
	C1	8	0	8	5	8
Nicotine 0.05 mg/mL	A2	8	3	8	8	3
	B2	8	2	8	8	7
	C2	8	0	8	7	7
Nicotine 0.1 mg/mL	A3	8	0	8	2	1
	B3	8	0	8	2	5
	C3	8	0	8	2	5
Nicotine 0.2 mg/mL	A4	8	0	8	3	5
	B4	8	0	8	3	5
	C4	8	1	7	1	7

Table 2. 4 Solutions: Qualitative Observations

Solution	Qualitative Observations
Control Group (Instant Ocean)	Healthy spines and eyes, normal growth.
Low Concentration (0.05 mg/mL)	Curved Spines, Lack of movement, stunted growth.
Medium Concentration (0.1 mg/mL)	Lack of Movement, Dark eggs, Curved Spine, stunted growth
High Concentration (0.2 mg/mL)	Curved Spines, lack of movement.

Graphs:





Results

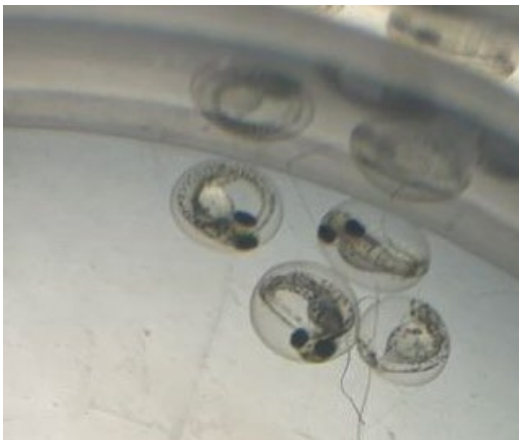


Fig. 2: Eggs within 0.2 mg/mL Nicotine solutions. Three healthy fetuses within eggs. A single underdeveloped egg in the bottom right.

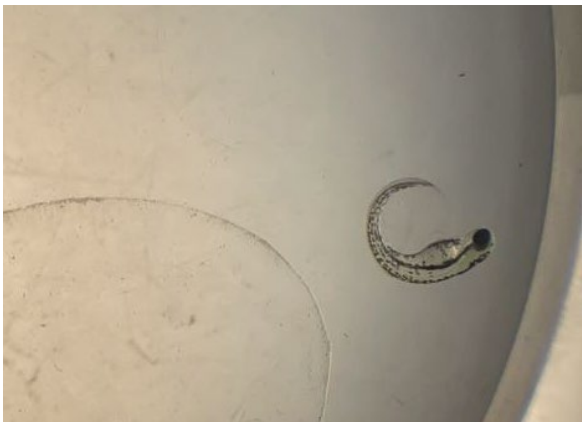


Fig 3: Deformed Spine Fish from 0.1 mg/mL nicotine solution.

Fig. 4: Deformed hatched Zebra Fish on the left compared to a functioning hatched Zebra Fish on the right from the 0.2 mg/mL nicotine solution.

In this experiment the research showed the effects of nicotine on the development of zebrafish. In order to see the effects of nicotine on the development of zebrafish, we created a control group using instant ocean in three wells with eight eggs in each well, the eggs in these wells were not impacted developmentally. This data was compared to the three wells we made for each of the three nicotine concentrations: 0.05 mg/mL, 0.1mg/mL, and 0.2mg/mL. Each well had eight zebrafish eggs which were observed over a 72 hour period to find the impact of nicotine on the zebrafish development.

Data was taken every 24 hours on each of the 12 wells and the 96 eggs. After 72 hours the instant ocean solution eggs were alive with no developmental issues. In the 0.05 mg/mL nicotine solution, 23 out of 24 eggs had hatched but 7 out of 23 zebrafish hatched died. We did not see any developmental issues in the zebrafish from the 0.05mg/mL nicotine solution group, the zebrafish were able to move and had healthy spines. In the 0.1mg/mL nicotine solution group,after 72 hours only 6 out of 24 eggs had hatched, leaving 13 out of the 24 zebrafish dead. The zebrafish that did hatch in the 0.1mg/mL nicotine solution suffered some developmental issues, specifically in their spine leaving a few deformed and unable to move. In the 0.2mg/mL nicotine solution group, after 72 hours only 7 out of 24 eggs had hatched and 7 had died. Many of the zebrafish that did hatch were left deformed, with misshapen spines and the inability to move past twitching. The eggs were also left deformed with multiple underdeveloped fetuses. Overall the most contrast from the constant group was seen in the 0.1mg/mL nicotine solution.

Discussion:

The alternative hypothesis which states that an increase in Nicotine will negatively affect a Zebrafish's development is supported by the lab. Both qualitative and quantitative data support the claim. The physical qualities of the control group zebrafish were much healthier compared to those of the different nicotine concentrations. The control group had healthy spines, normal eyes, and normal growth. In all three nicotine concentrations, the hatched fish had curved spines, stunted growth, and lack of movement. Stunted growth was also present in quantitative data, as in the control group 16 of the 24 hatched, and in the lowest nicotine concentration, 23 of the 24 hatched. However, in the 0.1 mg/mL nicotine solution only 6 of the 24 hatched, and in the 0.2 mg/mL nicotine solution only 7 of the 24 hatched. Similarly, in the 0.05 mg/mL solution 7 fish died, in the 0.1 mg/mL solution 13 fish died, and in the 0.2 mg/mL solution 5 fish died. Both the

qualitative evidence and quantitative evidence clearly support the concept of nicotine negatively impacting zebrafish, as their physical attributes were negatively changed as well as the survival and hatch rates.

Considering zebrafish gives a good insight into humans without experimenting on humans, the defects and negative impacts seen on the nicotine-impacted zebrafish eggs demonstrate the negative impacts nicotine can have on a fertilized human egg. The zebrafish data presents negative nicotine impacts such as birth defects, prenatal death, and infant death, and human babies exposed to nicotine while in the womb experience defects, prenatal death, and even after birth when infants have SIDS. The Zebrafish development directly mirrors that of human infants as well when both are exposed to nicotine.

There were several possible sources of error and limitations in this project. One possible source of error could be that the lab group missed one of the dead fish at 24 hours post-fertilization, making the number that survived after the first waiting period much higher than expected in the nicotine concentrations. Another source of error was when one of the pipettes previously placing 0.2 mL/mg nicotine solution was used to place 0.05 mL/mg solution into one of the wells. This was realized and the pipette was changed, but one of the wells of zebrafish in the lower concentrations was exposed to higher concentrations through the transferring of the pipette, meaning the data could be slightly inaccurate. A limitation was that the data taking was spread out in 24 hour periods, and the data would have been much more accurate if it were taken more frequently or for several more days.

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