The Impact of Nicotine on Zebrafish Embryos Development and Mortality

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Abstract

This research examines the effects nicotine could possibly have on the development and survival rate of zebrafish. The reason zebrafish were used in this experiment was to see the effects on developing embryos instead of using humans as test subjects. Nicotine is a chemical that is known to cause multiple health problems in the person using it, the people around them as well if the woman inhaling nicotine is carrying a child. Following that, in each well, there was a different concentration of nicotine, along with ten zebrafish embryos. Each set of embryos was exposed to different nicotine concentrations and examined after 24 hours, 48 hours, and 72 hours post-fertilization. In this experiment, the embryos that were exposed to higher amounts of nicotine suffered from abnormalities and even death. This experiment helps showed expecting mothers what may happen if they start or continue to smoke or inhale nicotine during pregnancy.

Introduction

It is a known fact that nicotine is a very addictive and dangerous chemical found in smoking devices. In the following experiment, zebrafish embryos were exposed to different amounts of nicotine. Zebrafish are great models for testing this chemical. According to Kurt Svoboda (2001), “Zebrafish, like humans have nicotine acetylcholine receptors”. Therefore, testing on zebrafish is safer than testing on humans, due to the speed they can produce higher amounts of offsprings for faster results.

Smoking is known to affect a person because of the chemicals one inhales while smoking. However, while pregnant, smoking also affects the child. Wickstrom (2007) states, “Women using smokeless tobacco have also been shown to give birth earlier than women not using tobacco, increasing the risk of preterm birth by a factor of 1.4%”. Nicotine is affecting the birth of children, being born early has a higher risk of birth defects and death at birth. Andrew Seaman (2013) found “babies of mothers who smoke may be born smaller or have impaired brain development”. This suggests that children of mothers who smoke are born small, this may lead to issues in weight and height. It also suggests that these babies may have brain issues.

In the following experiment, the mortality of zebrafish, as well as birth defects, were observed. Due to the increasing use of nicotine, while pregnant, it is important to be educated which the problem, it allows people to know how they affect their own children while pregnant. Therefore, if zebrafish embryos are exposed to increasing amounts of nicotine, then they will

have birth defects as well as possible death because nicotine has been known to affect unborn fetuses.

Materials & Methods

Materials:

- (40) Zebrafish Embryos
- (1) Stock solution of Nicotine (0.0, 0.05, 0.1, 0.2 mg/mL Nicotine)
- (5) 100 mL Breakers (one for dead embryos and waste, four for dilutions)
- (1) Sharpies
- (1) Embryos Media Solution
- (4) Large bore transfer pipette
- (1) Fine bore pipette
- (1) Multi-well plates
- (1) 28.5 °C incubator
- (1) Dissecting microscope
- Datasheet
- Gloves

Methods:

Gloves should be worn as a safety precaution to protect skin from chemical contact.

In the following experiment, observations were made to determine how nicotine affects the mortality and birth defects of zebrafish exposed to nicotine.

On the first day, the tray was labeled with the different amounts of nicotine in each well. The amounts labeled were 0.0, 0.05, 0.1, 0.2 mg/mL. Only the top four rows of wells were used. After labeling ten zebrafish embryos were placed in each well, 40 in total. The zebrafish that were chosen, were translucent to the naked eye, the embryos that were cloudy and white were considered dead and not chosen. After placing in the embryos, the excess liquid the embryos came in was removed using a fine bore pipette. Then, the tray was placed under the microscope to confirm there were ten live embryos in each well. Next, 1 mL of the different concentrations of nicotine solution was placed in it's designated well. The zebrafish were then examined under a dissecting microscope once more and pictures were taken. Lastly, they were placed in a 28.5 °C incubator for 24 hours.

On days two and three, the embryos and hatched zebrafish were examined. The old solution was removed as well as hatched embryo shells and obvious dead embryos. The new solutions in each well (still 1 mL of each concentration) were placed. The hatched zebrafish were then recorded, as well as how many were alive in embryos. The trays were then placed under a dissecting microscope for observations. The observations that were recorded were the movement of hatched zebrafish, how they reacted to a pipette being snuck upon them, and overall looks, body shape, and any apparent deformities in comparison to the control. Pictures of each well were taken daily to track the growth of the zebrafish. After each day of observations, the zebrafish were placed back in the 28.5 °C incubator.

The previous steps of observations of taking pictures and examining fish were repeated on day four. After observations, the living zebrafish were placed in a nursery tank with other living zebrafish. The dead embryos and fish were discarded. A chi-square analysis of the data was completed to ensure statistical significance.

Results

This study was designed to test the effects nicotine has on unborn fetuses. The test subjects used in this experiment were zebrafish embryos. Using the zebrafish embryos, data and observations were collected to determine how nicotine affects the mortality and birth defects of zebrafish. In figure 1 and 2 below, data shows how exactly the nicotine affects mortality and birth defects.

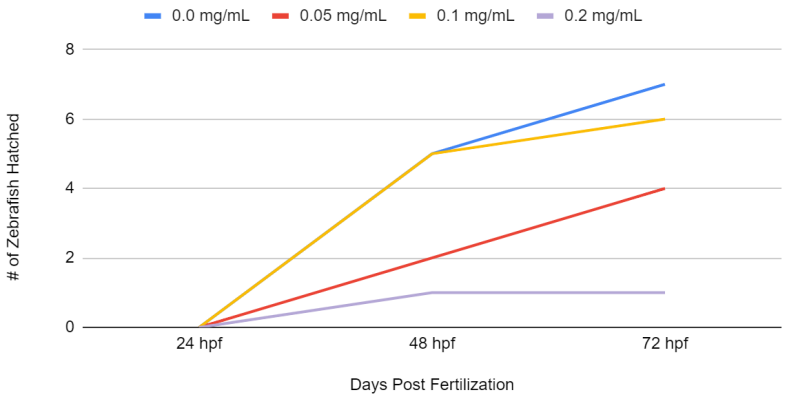
The dependent variable in this experiment was the mortality and hatch rate of the embryos. The independent variables in this experiment were the 0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL nicotine solutions.

The control in the experiment was the 0.0 mg/mL of nicotine. The controlled variables were the number of zebrafish embryos, the size of wells, and temperature (28.5 °C) in the incubator.

A chi-square analysis was created to test the independence of nicotine solutions and the mortality rate of zebrafish embryos. The chi-square value was 17.92. The null hypothesis was rejected using a critical value of 7.82 and 3 as a degree as freedom. This means the results given were not by chance, but these results were given because of the amounts of nicotine added to the embryos and the nicotine did affect the developing embryos.

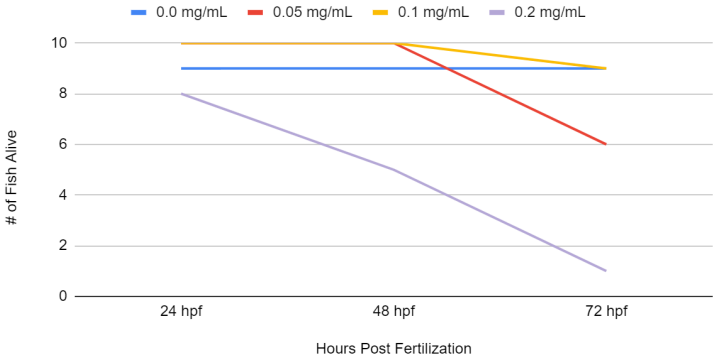
# of Zebrafish Hatched Overtime

Figure 1: shows number of zebrafish hatched over a 72 hour time span.



Mortality Rate on Zebrafish Overtime

Figure 2: shows mortality rate on zebrafish over 72 hour time span



It was found that embryos in the control solution (0.0 mg/mL) were most successful. In the end, all of these embryos were living and had no birth defects (fig. 3). Overall, as the amount of nicotine increased, the death of embryos and deformities increased. However, this was not a trend, some days would vary with the amounts of death. For example, after 24 hpf, three zebrafish died throughout the 40. 48 hpf, three more died and after 72 hpf, nine had died. Throughout observations, the zebrafish in higher concentrations were more likely to die. This is shown in figure 2. Even if embryos hatched, they did not move a lot and/or were curled up, assuming they contained birth defects (fig. 3).

On day two (24 hpf), no zebrafish had hatched, but a few had died in the embryo. One died in the 0.0 mg/mL well, along with two in the 0.2 mg/mL (fig. 2). Day three (48 hpf), five had hatched in the 0.0 mg/mL well, two hatched in the 0.05 mg/mL well, five hatched in the 0.1 mg/mL well, and none had hatched but three died in the 0.2 mg/mL well (fig. 2). On the last day (72 hpf), all embryos hatched in 0.0 mg/mL well, 0.05 mg/mL well, all embryos hatched but four died, 0.1 mg/mL well, all hatched but one died, and 0.2 mg/mL one fish was hatched alive (fig. 1).

Development of Deformities in 48 hpf

Figure 3: shows deformities among fish in different amounts of nicotine concentrations

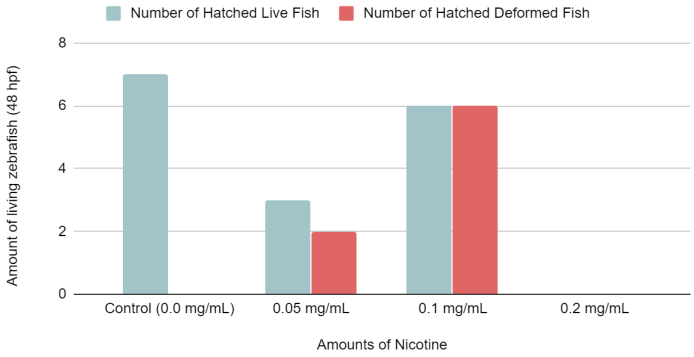


Table 1: Number of Embryos Hatched and Alive after 72 hpf: The table shows number of fish alive and hatched after the 72 hour post fertilization

Treatment	# of starting fish	24 hpf	24 hpf	48 hpf	48 hpf	72 hpf	72 hpf
Nicotine	40 fish total	#Hatched	#Live	#Hatched	#Live	#Hatched	#Live
0.0 mg/mL	10 fish	0	9	5	9	7	9
0.05 mg/mL	10 fish	0	10	2	10	4	6
0.1 mg/mL	10 fish	0	10	5	10	7	9
0.2 mg/mL	10 fish	0	8	1	5	0	1

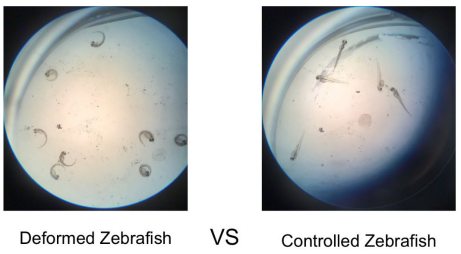


Figure 5: Deformed and Controlled Zebrafish This shows deformed zebrafish in 0.1 mg/mL well and controlled zebrafish

Discussion

The hypothesis in the experiment was if zebrafish embryos are exposed to increasing amounts of nicotine, then they will have birth defects as well as possible death because nicotine has been known to affect unborn fetuses. According to the research, nicotine use in humans causes birth defects to unborn fetuses, premature birth, and possible death. Zebrafish embryos were used in the experiment due to the fact that humans and zebrafish both have nicotine acetylcholine receptors.

When conducting research it was found that the higher the nicotine, the more deformities. In the 0.0 mg/mL well none of the hatched fish had deformities compared to the rest of the wells. More than half of the hatched fish were deformed in the 0.05 mg/mL well, all of the hatched fish were deformed in the 0.1 mg/mL well, and no embryos were alive in the 0.2 mg/mL well to be deformed. This shows that if there is a nicotine substance, the embryo will be affected. Previous research mentioned that birth defects were most common to occur.

It was also shown that death was more common among the higher concentrations. The most obvious death was in the 0.2 mg/mL well. At the end of the 72 hr experiment, no fish survived in that

well. The 0.1 mg/mL and 0.05 mg/mL wells still had all 10 alive, but barely any had hatched. In the 0.0 mg/mL well nine of the fish were alive, hatched, and healthy. When eggs were picked for the 0.0 mg/mL well, one was cloudy under the microscope which leads to death. This could have been avoided by choosing better eggs at the start. Even though data still showed that higher concentrations lead to death and deformities in developing embryos. Previous research also shows that death is likely when nicotine is contained, especially high amounts. One limitation to our experiment was that only one experiment was conducted and the sample size was small. To improve this, more than one experiment should be conducted with a larger sample size.

In conclusion, the outcome of this experiment was no surprise and not by chance. Going back to the chi square analysis, the outcome was 17.92. Previous research supports the results; nicotine does have a dramatic effect on unborn fetuses and embryos. Both humans and zebrafish demonstrate these effects. However, this educational study answers the questions of how nicotine affects an unborn organism. A question for further research would be; what would be the cutoff for lower concentrations, how much nicotine can an embryo intake without facing birth defects or death.

References & Literature Cited

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