

The Effect of Nicotine on Zebrafish Embryo Development

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Abstract

Over 1000 unborn children die every single year due to their mother smoking while pregnant (American Pregnancy Association, 2017). Nicotine is a very harmful and deadly substance that is consumed while smoking, vaping, etc. (National Institute of Drug Abuse, 2020). It carries through the bloodstream and if pregnant, will go straight to the embryo in the womb (Cleveland Clinic, 2020). The study being conducted is testing to see how nicotine affects the development of zebrafish embryos. It was expected that there would be a correlation between a higher concentration of nicotine and the survival rate of the embryos. Fish embryos were exposed to different concentrations of nicotine and data was recorded on what percent of the embryos survived in each environment. Essentially, what was found was that with an increase of the concentration of nicotine, the more zebrafish that were found dead. This further supports the idea of just how harmful nicotine affects the development of embryos in different organisms .

Background Information

Why Fish?

A model organism is a non-human species that is used in the laboratory to help scientists understand scientific processes (Yourgenome, n.d). Model organisms are used in scientific research because they are easy to maintain and breed in a laboratory setting and allow scientists to study things about humans using other organisms. Zebrafish are good models for studying development because they grow at an extremely fast rate, developing as much in a day as a human embryo does in a month (Yourgenome, n.d). This makes them a good indicator for human development since they can replicate it but at a much faster rate. Zebrafish also share 70 percent of their genes with humans, proving for better results in the experiment. (Yourgenome, 2019)

Your Variable

Studies show that over 1,000 babies die each year because their mothers smoked while pregnant (American Pregnancy Association, 2017). Nicotine is a highly addictive substance found in products such as cigarettes, juul pods, vape pens, etc. Nicotine users are more prone to diseases such as cancer, heart disease, stroke, lung diseases and diabetes (Centers for Disease Control and Prevention, 2018). It is a very harmful and deadly substance. It is important to note that what a mother consumes during pregnancy is the same as what the baby consumes (KidsHealth, 2018). The nicotine, carbon monoxide, and numerous other poisons that are inhaled from a cigarette are carried through the bloodstream and will go directly to the baby (Center of Disease Control and Prevention, 2018). Smoking during pregnancy lowers the amount of oxygen available to the baby, increases the baby's heart rate, and increases the baby's risk of developing lung problems (WebMD, 2019). Scientists have also proven that

smoking during pregnancy may lead to premature birth, lower infant birth weights, birth defects and ultimately infant death.

The Investigation

The investigation is going to be looking more in depth on how varying amounts of nicotine affect the development of Zebrafish embryos. Using this information, a conclusion can be drawn on whether or not it, in turn, affects human embryos in a similar way. This data is important in strengthening the argument that nicotine is a harmful substance, especially during pregnancy. The hypothesis is that when exposed to the highest concentration of nicotine, survival rates will go down by a significant amount.

Participants

On the week of November 4th, 2019, Honors Biology Students from Greendale Highschool participated in an experiment that tested the effect of different substances on embryological development of Zebrafish. Students could choose between nicotine, caffeine and ethanol. Students received the embryos from the school of freshwater science on Monday, November 4th and began testing the following Tuesday. The eggs were placed in an instant ocean to which then they were distributed in wells and used in various different experiments that the students conducted.

Materials

1. 12 wells that can contain about 75mL of liquid.
2. One small pipette for liquid
3. One larger pipette for the eggs.
4. 4 bottles for containing the different concentrations of nicotine and control group
5. Small graduated cylinder for creating nicotine concentrations
6. Large graduated cylinder for creating concentrations
7. 72-120 Zebra fish eggs
8. Microscope for examining eggs (optional)

Design

In the experiment the independent variable is the concentration of nicotine used on the eggs and the dependent variable is the amount of Zebrafish that survive. Each well will contain the same number of eggs and the same amount of liquid. There will be 3 wells with control embryos that will have zero nicotine exposure, 3 wells exposed to 0.05 mg/mL of nicotine, 3 wells exposed to 0.1 mg/mL nicotine and 3 wells exposed to 0.2 mg/mL nicotine. Data will be kept on the amount of eggs that hatch and the amount of embryos that live in each well over intervals of 24 hours .

Procedure

1. Gather all materials for lab
2. Distribute 6-10 eggs in each well using the large pipette
3. Drain any excess liquid from each well using the smaller pipette , make sure no eggs are displaced during this process.
4. Create .05mg/ml, .2mg/ml, .1mg/ml concentrations of Nicotine solutions. Use the small graduated cylinder for precise amounts and the larger ones for the amount of instant ocean.
5. Add Nicotine solutions/control solution to wells and ensure that each well is about halfway full with solution
6. Count the number of fish hatched and alive over intervals of 24 hours. This can be done by putting the wells under a microscope. If they are black under the microscope they are dead. If they are white under the naked eye, they are also dead.
7. Replace solution over intervals of 24 hours
8. Be careful and mindful with the substances. Use your common sense. (UWM,n.d)

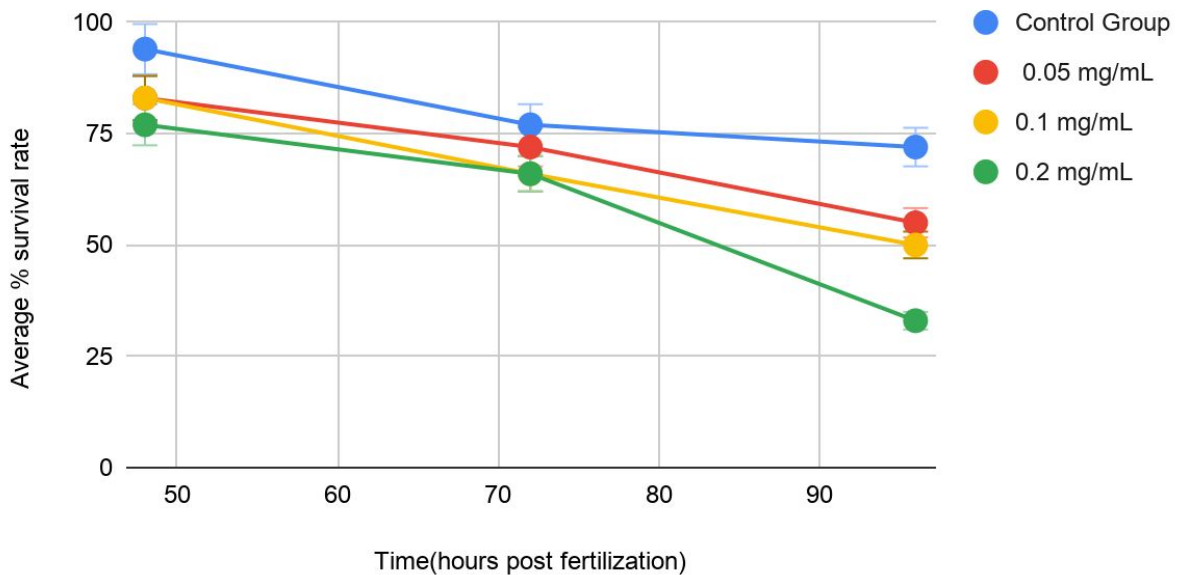
Results

Summary of Results

The experiment tested the effect of Nicotine on the development of Zebrafish embryos. 72 zebrafish eggs were distributed among 12 different wells. There were four total treatments with a control treatment and three treatments with varying levels of nicotine. The tests taken showed that with an increase of nicotine, an increased number of Zebrafish died during the experiment.

Tables and Figures

The percent survival rate of zebrafish exposed at different levels of nicotine over time.



This Graph shows the average percent of zebrafish that survived per treatment at intervals of 24 hours post fertilization.

Time(hours post feratalzarion)	Control Group	0.05mg/mL	.1mg/mL	.2mg/mL
48 hours	17/18	15/18	15/18	14/18
72 hours	14/18	13/18	12/18	12/18
96 hours	13/18	10/18	9/18	6/18

This table shows the total amount of zebrafish alive in each group



This photo shows a zebrafish 96 hours post fertilization. Compared to the control group, you can see a clearly underdeveloped egg, with deformities in the back, and only one formed eye.



Zebrafish in the control group 96 hours post fertilization. Healthy spine, with two full eyes.

Statistical Findings

It was found in the experiment that after 96 hours, in the control group, 75% of the zebrafish lived. However, the group that was exposed to the largest concentration of nicotine had a 33% survival rate. This statistical test was taken to identify if varying levels of nicotine discreetly affect the development of zebrafish in seeing whether or not they make it to adulthood. The significance in doing this test is that these numbers are purely quantitative and aren't objective from person to person. Also, this difference in survival rate is significant in showing the effect that nicotine has on the development of the zebrafish which makes it a good indicator for the experiment in showing the effect.

Discussion

Importance of the Topic

This study essentially highlights how nicotine affects the development of zebrafish which correlates towards human embryos. The significance of this is that nicotine can ultimately affect the development of (UWM, n.d). This research is geared towards discouraging mothers from smoking in the essence of preserving their baby. This is undeniable evidence because nicotine affects development and exposure is linked to not developing as much and ultimately death in human embryos. (American Pregnancy Association, n.d)

Importance of the Findings

The findings are important since they show a clear relationship between higher death rates among eggs that were exposed to nicotine compared to those that were not. This supports the original hypothesis that the higher the concentration of nicotine the eggs were exposed to, the higher the death rate was among those eggs. This is proven with only 33% of the eggs being alive in the 0.2 mg/mL nicotine solution at 96 hour post fertilization, compared to 77% survival rate among the eggs in the control group after 96 hours post fertilization.

There were many limitations with the lab, including not being able to put the solutions into the well until 48 hours post fertilization. In addition, some oddities occurred in a couple of the wells, with the total number of fish being slightly uneven from day to day. Furthermore, time constraints made some days harder to properly observe the zebrafish development. Overall, the data is still significant enough to believe that these results are repeatable.

Future research should continue to explore the link between zebrafish and human embryos, and see if these results can have a direct comparison. In addition, the different types of deformities that are caused when nicotine is found in a zebrafish embryo. These questions would both give a clear understanding of the true effect that nicotine has on zebrafish, and potentially humans .

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