The Effects of Nicotine on the Hatch Rate of Developing Zebrafish Embryos

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Abstract

Studies in the past have shown that nicotine can affect the hatch rate and survival rate in zebrafish embryos. It is important to know the effects nicotine has on developing embryos because human embryos are very similar, they have similar genomes. This means that it is possible to make conclusions on what will happen if a pregnant woman smokes. The purpose of this experiment is to help educate on the negative effects nicotine can have on zebrafish embryos and human fetuses The embryos were raised in four different nicotine solutions: 0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL of nicotine. This experiment took place over the course of five days, data was collected after every day. The data showed that the wells with higher concentrations of nicotine had a higher hatch rate. This data proves that nicotine is increasing the risk of stillbirths in mothers who smoke. The data that was collected throughout the experiment has shown that nicotine does cause premature birth and stillbirths, it also decreases the survival rate of the zebrafish embryos. All of this research concludes that the nicotine would have the same effect on a developing child then it would on a zebrafish embryo since their genetic material is similar. Nicotine increases the chance of an underdeveloped child, it should be seen as a warning to mothers who smoke.

Introduction

The purpose of the research is intended to inform readers and the general public on the effects that nicotine has on the weight and development of both zebrafish and human fetuses. The purpose of this report is to also raise awareness for soon-to-be mothers to stop smoking for the safety and health of their children.

According to Chaturvedi et al. (2015), “The two main compounds suspected of causing harmful effects on the developing fetus during pregnancy are monoxide and nicotine.” Mothers that are pregnant or nursing think if they only smoke one or two cigarettes a day it won’t affect the baby.

The nicotine doesn’t just affect the mother, it also affects the unborn child. “Nicotine crosses the placenta reaching levels in the amniotic fluid and fetus that exceed those of the mothers. Nicotine is also measurable in the breastmilk of smoking mothers as well as mothers passively exposed to cigarette smoke. The risk and magnitude of low birth rate is related to the number of cigarettes smoked during pregnancy.” Said by Chaturvedi et al. (2015). The nicotine levels in a fetus are higher than the levels of that in the mother.

“Our review confirms a dose-response effect of maternal smoking in pregnancy on risk of stillbirth.” from Marufu, T. C., Ahankari, A., Coleman, T., & Lewis, S. (2015). This is showing that there is a correlation between smoking while you are pregnant and having a higher risk of a stillbirth. The more nicotine you have present in your body (the amount of cigarettes), the higher the probability of a stillbirth.

If the zebrafish are exposed to Nicotine, then the hatch rate will be reduced because nicotine is known to increase the amount of stillbirths and miscarriages in human fetuses.

Materials and Methods

The materials included 4-100 mL beakers filled with the different nicotine concentrations (0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL), multiple fine bore and large bore pipettes, 40 live zebrafish embryos, a dissecting microscope, a multi well plate, an incubator, and a data sheet.

On the first day, 40 live zebrafish embryos were obtained. The cover plate of the multi-well plate was labeled with the concentration of nicotine in each well, names, and “nicotine” with a sharpie. Ten embryos were placed in each of the four wells using a large bore pipette. Next, the residual fluid was taken out of each well using a fine bore pipette to make sure the embryos wouldn’t get extracted along with the fluid, and replaced with the nicotine concentration designated to each of the wells. This was done by extracting the nicotine solution up through the large bore pipette and carefully removing the right amount down to 1mL to make sure exactly 1 mL was taken out. 1 mL of the control, which is 0.0 mg/mL, was placed using the pipette in the first well. In the second well, 1 mL of 0.05 mg/mL was placed with another large bore pipette . The third well had 1 mL of 0.1 mg/mL put into it with a pipette. Lastly, 1 mL of 0.2 mg/mL was

placed into the fourth well. After the embryos were put into the well, they were put under the microscope and pictures were taken to record what they looked like on day one, and used for future reference. Once the pictures and observations were made, the embryos were put into an incubator to make sure they would stay alive for 24 hours.

On day two, the plate was removed from the incubator, and the dead embryos were removed and put into the waste beaker. Then, the hatch rate of each well was recorded. The hatch rate included the amount of live eggs and hatched eggs. The hatch rate was measured by observing the zebrafish after every day through the dissecting microscope and recording on the data sheet the amount of zebrafish embryos hatched in each well. Having the zebrafish completely out of the embryo was considered hatched. The well whose zebra fish hatched in the shortest amount of time was the well with the highest hatch rate. Pictures were taken of the zebrafish in each well. Then, like day one, the steps were repeated and the fish were given fresh nicotine solution of the same amount. Lastly, the tray was returned to the incubator which was kept at 28.5 degrees Celsius.

On day three/four, it was repeated what occurred on day two by removing the dead zebrafish from the plate using the fine bore pipette. Then the nicotine solution was replaced, observations, hatch rate, and pictures were taken of the remaining zebrafish. After all of this, the zebrafish were taken from the tray to the incubator.

On day five, all of the observations and data were recorded again like all of the previous days. After writing down all of the information that was needed, the rest of the nicotine solution was extracted from the wells, and the remaining zebrafish were put into the nursery using a large bore pipette. During the experiment the following safety precautions were taken. Fine bore pipettes were used in order to keep the fish safe and not to damage them. The nicotine solution was replaced every 24 hours to make sure the fish had a clean living environment. The fish were kept in an incubator after they were observed to make sure they stayed warm. Gloves were worn when measuring nicotine solutions to prevent nicotine exposure.

A Chi Square analysis of the data was completed to ensure statistical significance.

Results

The hypothesis was if the zebrafish are exposed to nicotine, then the hatch rate will be reduced because nicotine is known to increase the amount of stillbirths and miscarriages in human fetuses. The purpose of this was to not only raise awareness to future mothers but also to inform readers on the effects that nicotine could have on zebrafish and human fetuses. The experiment was set-up this way with the varying amounts of nicotine so that we could record how the zebrafish reacted in the different environments, and if the concentration had any affect on the development of the embryos. The independent variable in the experiment was the concentration of the nicotine in each well. The dependent variable was the hatch rate. The control was the 0.0 mg/mL solution. Controls in the experiment were the size of the wells, temperature of water/ nicotine solution and the amount of new water/solution each day.

The independent and dependent variables are related because the higher concentration in the well results in a higher hatch rate. The zebrafish in the lower nicotine concentration hatched slower, but were much more developed and healthy looking.

On the second day, one embryo in the 0.0 mg/mL solution was hatched. Three embryos were hatched in the 0.05 mg/mL solution. One zebrafish embryo was hatched in the 0.1 mg/mL well. In the last well (0.2 mg/mL) two embryos were hatched.

This experiment was planned in this way so the results could easily be seen and observed. Using the tray was a tidy and organized way to keep the nicotine solutions separate. The varying amounts of nicotine were used to simulate and show the effects of nicotine on developing fetuses.

The Chi Square analysis for the hatch rate, the hatch rate data had a Chi square value of 5.45 which was less than the critical value of 7.82 using three degrees of freedom and a P-value of 0.05. That showed that the null hypothesis should be accepted since it was less than the critical value, which means the data could have happened by chance. There wasn't a statistically significant correlation between the hatch rate and nicotine concentration.

Treatment	# of starting fish	24 hours post fertilization		48 hours post fertilization		72 hours post fertilization		96 hours post fertilization	
		#Hatched	#Live	#Hatched	#Live	#Hatched	#Live	#Hatched	#Live
0.0mg/mL	10	0	8	1	8	8	8	8	8
0.05mg/mL	10	0	7	3	7	7	5	5	0
0.01 mg/mL	10	0	8	1	7	7	7	7	6
0.2mg/mL	10	0	7	2	7	5	4	4	0

Figure 1: This raw data table above is showing how the nicotine affects the zebrafish hatch rate. The wells with the higher nicotine concentrations had less embryos alive after the 96 hour period, they also had more hatched in a shorter amount of time.

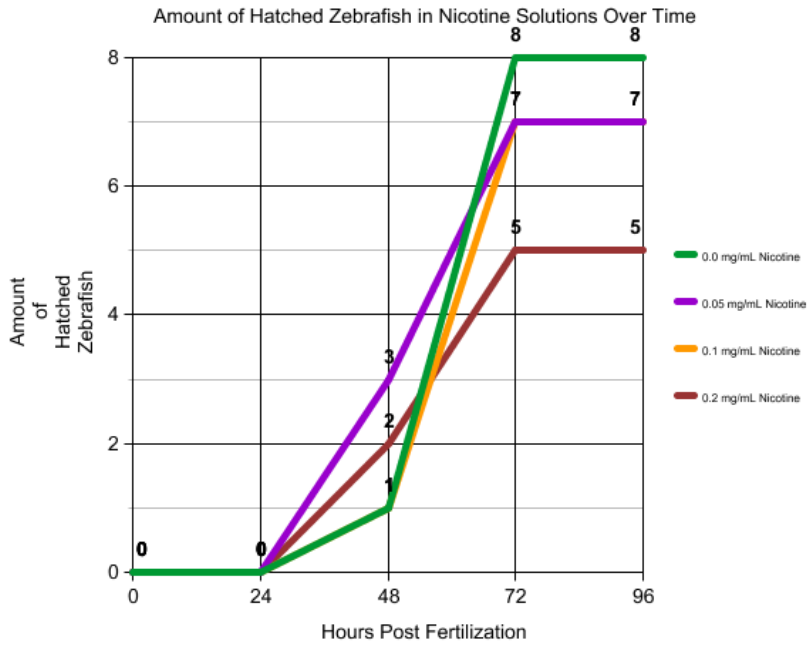

Figure 2: The line graph shows the relationship between the amount of nicotine present in the wells and the amount of hatched zebrafish. The wells with higher nicotine concentrations had a higher hatch rate with fewer hatched.

Discussion

According to the research, nicotine is one of the two main chemicals that causes birth defects, premature birth, and SIDS (sudden infant death syndrome). Also that the nicotine levels in a fetus are higher than that of the mother, and the nicotine levels are

passed through nursing after the baby is born. Zebrafish also chose to spend more time on the side of the tank that was polluted with nicotine.

Faster doesn't necessarily mean better. In humans when babies are born too early they have development issues and a greater chance of dying prematurely. The same applies to zebrafish on day 4, eight zebrafish in well one (0.0 mg/mL) were still alive. In well 2 (0.05 mg/mL) 0 zebrafish were alive. Well 3 (0.1 mg/mL) had 6 Living zebrafish. 0 zebrafish were still alive in well 4 (0.2 mg/mL).

After twenty-four hours of fertilization none of the embryos hatched. Then the number of hatched embryos increased the most in well 2 (0.05 mg/mL) after 48 hours post fertilization. 72 hours after fertilization well 1 (0.0 mg/mL) had the most amount of hatched embryos. 96 hours post fertilization well 1 was still in the lead with the number of hatched embryos.

The null hypothesis was accepted because the hatch rate data only had a chi square value of 5.45 which was less than the 7.82 needed to reject the null hypothesis. This means that the hatch rate data that was recorded could have been by chance, or there wasn’t enough data to prove our hypothesis.

One thing we could do to improve this experiment is to increase the sample size and add more variations of nicotine to get more data and reject the null hypothesis.

Some things that are still unanswered are how the heart rate is affected with the varying nicotine concentrations and if it has anything to do with the birth and death rates.

What was learned from this experiment was that nicotine does affect the hatch rate in zebrafish embryos, since nicotine is known to cause premature births and stillbirths.

You can see the embryos in the nicotine solution hatched quicker than the embryos in the lesser amount of nicotine solutions. This data fails to support the hypothesis.

The initial hypothesis was proven wrong in the experiment. (In figure 2 above), the embryos in the more concentrated hatched first.

In the future the experiment could be improved by using a larger sample size, over a longer period of time and with more and different nicotine concentrations.

One significant thing observed in the results was that the number of zebrafish that lived wasn’t consistent to the concentration. The only thing that was directly affected by the concentration was the hatch rate, the higher concentrations had a higher hatch rate.

In conclusion, nicotine speeds up the hatch rate but their chance of survival decreases.

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