

The Effect of Nicotine on Zebrafish

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Abstract:

In this experiment, the effects of nicotine on pregnant women were demonstrated by using zebrafish as a model to show the effects of nicotine exposure to their eggs and hatchlings. We tested the zebrafish by exposing them to three different amounts of nicotine (0.05 mg/ml, 0.1 mg/ml, and 0.2 mg/ml), and observed how the zebrafish eggs would react when exposed to the nicotine. The eggs reacted in multiple different ways including delayed hatching, physical deformities of the hatched zebrafish, and premature deaths of the fish. We found that a little nicotine affected the fish in small ways such as causing it to have a slightly curved spine or it having a poor functioning nervous system. However, if the fish were exposed to larger amounts of nicotine, there were bigger impacts such as weakness, making it difficult for the eggs to hatch. The significance of these results were that it demonstrated how it could be dangerous and harmful for the unborn child of a pregnant mother. Therefore, it could be said that nicotine should not be consumed during pregnancy, even if it is a small amount.

Introduction:

The chairman of the Obstetrics and Gynecology at Providence Hospital, Robert Welch, stated that “smoking cigarettes is probably the No. 1 cause of adverse outcomes for babies” (Woolston, pg 1). There are many reasons why some babies may have issues when they are born. However, women who smoke while they are pregnant has been one of the main causes of babies having health issues as they develop. If nicotine is consumed by a pregnant woman, then their baby could develop many effects such as lung problems, abnormal weight, increase in heart rate, a lower amount of oxygen received by, increased chances of he/she being stillborn or miscarried, an increased chance of getting Sudden Infant Death Syndrome, and various birth defects (Pagano, pg 1). However, 15 to 25 percent of women still smoke during pregnancy, despite these

consequences (Wickstrom 2007). In addition, secondhand smoke, a combination of smoke exhaled by the smoker and the smoke from the end of the cigarette, can have multiple effects on both the unborn baby and mother. Regular exposure to secondhand smoke while pregnant can lead to a higher chance of miscarriage, stillbirth, and other pregnancy complications. In an effort to provide further education about the harmful effects of nicotine, this lab will use zebrafish as a model to demonstrate the effects of increased nicotine exposure on the growth and development of organisms. After researching, we hypothesized that if zebrafish eggs are exposed to nicotine, then the fish will have an increased chance of having health issues such as weakness or a curved spine because over 4,000 dangerous chemicals in nicotine go directly from the zebrafish bloodstream to the baby.

Materials:

- Dissecting microscope
- 12 well plate
- Instant Ocean Solution
- Methylene blue
- 3 concentrations of nicotine (.05 mg/ml, .1 mg/ml, .2 mg/ml)
- 2 Gloves per person
- 1 Safety goggle per person
- 1 large pipet for eggs
- Small pipets for everything else

Methods:**Day 1**

1. Grab Safety goggles
2. Label the wells (A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3)
3. Add 10 zebrafish eggs to each well
4. Add 2ml of Instant Ocean to A1-A3 wells
5. Add 2ml of 0.05mg/ml of nicotine to B1-B3
6. Add 2ml of 0.1 mg/ml of nicotine to C1-C3
7. Add 2ml of 0.2 mg/ml of nicotine to D1-D3
8. Dip a pipet in methylene blue, than in wells A1-A3
9. Dip a new pipet in methylene blue, than in wells B1-B3

10. Dip a new pipet in methylene blue, than in wells C1-C3
11. Dip a new pipet in methylene blue, than in wells D1-D3
12. Place the well plates in an incubator at 28.5 degrees C

Day 2-4

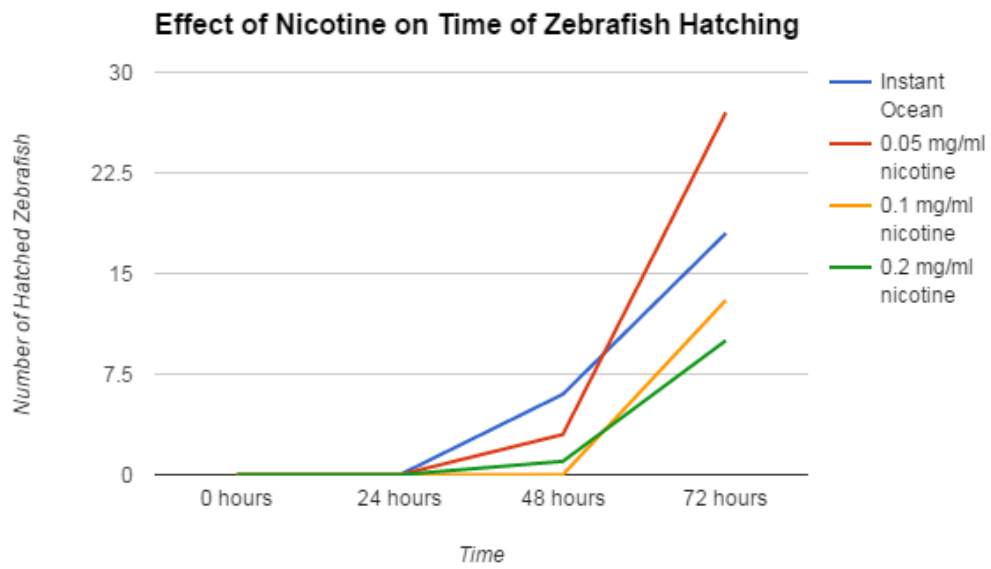
1. Count the number of eggs hatched and alive in wells A1-D3.
2. Remove the dead eggs with a pipet, put them in a separate beaker, and wash them down the sink.
3. Remove the old solution from each well with a pipet.
4. Put 2ml of Instant Ocean in each wells A1-D3.
5. Put 2 ml/mg of 0.05 nicotine in B1-B3.
6. Put 2 ml/mg of 0.1 nicotine in C1-C3
7. Put 2 ml/mg of 0.2 nicotine in D1-D3
8. Dip a pipet in the methylene blue and put it in A1-A3
9. Dip a new pipet in the methylene blue and put it in B1-D3
10. Take visuals of zebrafish and observe their features or characteristics (ex: curved spine, one eye)
11. Place the well plates in an incubator at 28.5 degrees C

Effects of Nicotine on Zebrafish Eggs

Treatment	Well #	# of Starting Fish	24 hours post fertilization		48 hours post fertilization		72 hours post fertilization	
			#hatched	#live	#hatched	#live	#hatched	#live
Instant ocean	A1	10	0	9	0	9	8	8
Instant ocean	A2	10	0	8	1	8	3	7
Instant ocean	A3	10	0	10	5	9	7	8
0.05 mg/ml nicotine	B1	10	0	10	0	10	9	10
0.05 mg/ml nicotine	B2	11	0	10	2	10	10	9
0.05 mg/ml nicotine	B3	10	0	10	1	10	8	8
0.1 mg/ml nicotine	C1	10	0	9	0	8	0	8
0.1 mg/ml nicotine	C2	10	0	9	0	9	4	9
0.1 mg/ml nicotine	C3	10	0	9	0	9	9	9
0.2 mg/ml nicotine	D1	9	0	9	1	8	9	9
0.2 mg/ml nicotine	D2	10	0	10	0	10	1	10
0.2 mg/ml nicotine	D3	9	0	9	0	9	0	8

Quantitative and Qualitative Data of the Features of ZebraFish

	Quantitative Data	Qualitative Data
A1	8 hatched on the third day, 2 died in total, all 8 have typical features	All zebrafish hatched on the third day and displayed typical features (two eyes, straight spine)
A2	1 hatched on the second day, 2 hatched on the third day, 3 died in total, all 7 have typical features	All zebrafish display typical features
A3	5 hatched on the second day, 2 hatched on the third day, 2 died in total, all 8 have typical features	All zebrafish display typical features
B1	9 hatched on the third day, 0 died, 2 have 1 eye, all 10 have nerve damage	A couple have a singular eye, and all have nerve damage
B2	All 10 hatched on the third day, 1 died with a curved spine and is significantly smaller, 9 have nerve damage	All have nerve damage, a singular fish has a curved spine and is significantly smaller
B3	One hatched on the second day, and seven hatched on the third day. Two died on the third day.	One has a bent spine in the spine with no eyes. Another has a curved spine. Another is shorter than the rest and has fewer lines.
C1	On the first day, 1 fish died. On the second, another died.	All of them have two in eyes in their shell, but are not hatched or responsive to movement.
C2	One died on the first day. On the third day, 4 fish hatched.	All hatched fish have a normal spine. Only one has one eye. All of the rest have two eyes.
C3	On the first day, 1 fish died. On the third day, all 9 fish alive hatched.	All look normal, but are too weak to hatch.
D1	All are alive, but only one hatched	May be too weak to hatch. All look normal
D2	All are alive, and on the third day, 1 fish hatched.	The one that hatched, is not respondent and their is a slight curve to its spine. May be too weak to hatch.
D3	Only 1 fish died in total out of the 9. This happened on the third day.	All have two eyes, but may be too weak to hatch.



Graphs



Results:

The main focus of this experiment was to find out how nicotine can affect a zebrafish egg as it develops. In order to do this, we put 0.05 mg/ml of nicotine in three wells with ten zebrafish eggs, 0.1 mg/ml of nicotine in three other wells with ten zebrafish eggs, and 0.2 mg/ml of nicotine in the last three well also with ten zebrafish eggs. We also had three control wells which were not affected in any way and were only given a little bit of Instant Ocean. In this experiment, our goal was to collect information on these 3 things: number of living fish, number of hatched fish in each individual well and the physical development of the hatched fish.

After collecting data after the first twenty four hours, we found that there were very few changes to the eggs. There were little to no deaths of any of the eggs and none that had hatched. After forty eight hours, we found that a fair amount of eggs from the control group had hatched

and little to no eggs had hatched from the groups that had nicotine added to them. Also, there were a few deaths here and there, but overall there were not any large amounts of deaths in a certain group. From what we could tell, the zebrafish (hatched and not hatched) all displayed typical and normal features. After 72 hours you can see a clear trend in hatch rate comparing the control and low concentration to the medium and high concentration. We found that after the concentration of nicotine increased past .05 mg/ml, it took longer for the zebrafish eggs to hatch. Even though the zebrafish exposed to 0.05 mg/ml of nicotine had the highest survival rate after 72 hours, the amount of nicotine didn't seem to have a large impact on the survival as all four groups were within four living zebrafish of each other. However, there were a few health issues and deformities that we found with the fish. First, we found that the fish that had been exposed to nicotine had a damaged nervous system because they didn't react when we touched the water near them. We also found that many of the fish that had been exposed to the 0.1 mg/ml of nicotine had curved spines, and some had only one eye (see fig. #3 for an example of a zebrafish with a curved spine). Most of the fish that had been exposed to 0.2 mg/ml of nicotine displayed normal features. This displays that nicotine did have effects on the zebrafish eggs, whether it be physical deformities or an effect on their hatch rate.

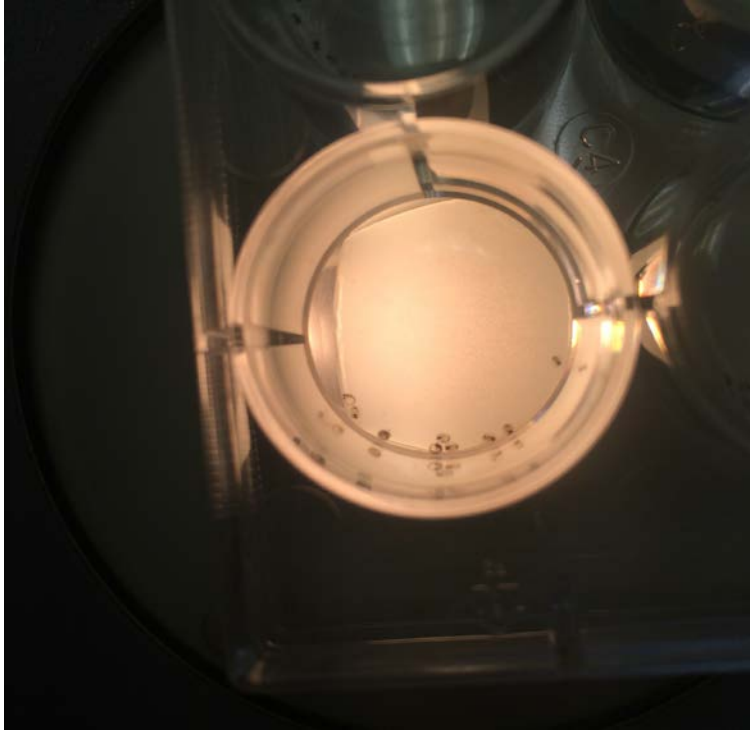


Figure #1

Well D3, 0.2 mg/ml nicotine, Day 3

In this visual, you can see the zebrafish eggs at the bottom of the well. The third egg from the left is an example of a dead egg from well D3. This egg is dead due to the blackness and the deformation of the zebrafish inside. All of the rest of the eggs in this picture are alive and have normal features.

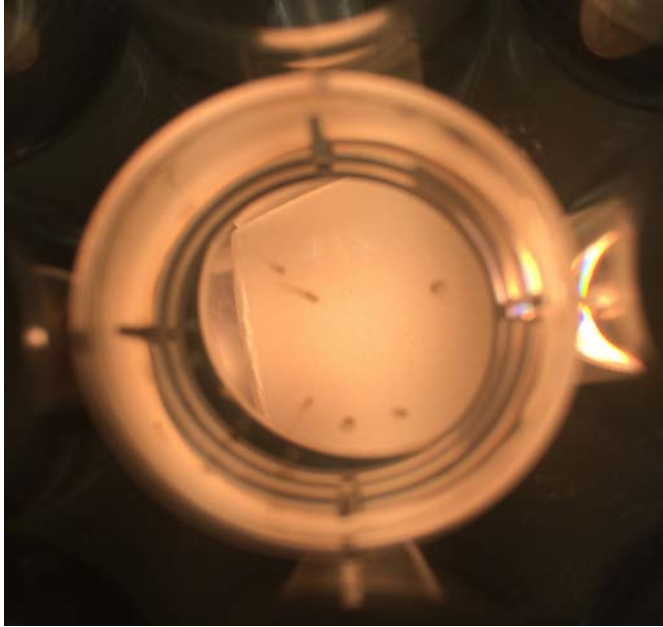


Figure #2

Well C2, 0.1 mg/ml of nicotine, Day 3

Although it may be difficult to tell, the first fish on the bottom left is an example of a hatched fish with normal features in well C2. The fish on the top left is an example of a hatched fish that has mostly normal features, but it is smaller than most of the hatched fish.



Figure #3

Well B2, 0.05 mg/ml of nicotine, Day 3

This image shows an example of a zebrafish with a slightly curved spine. Towards the bottom of the fish, the spine starts to curve slightly.

Discussion:

Our hypothesis, was that if zebrafish eggs are exposed to nicotine, then the baby will have an increased chance of having health issues such as weakness or a curved spine because over 4,000 dangerous chemicals in nicotine go directly from the zebrafish bloodstream to the baby, was supported by this experiment. Based on our findings from this lab with zebrafish eggs, we can come to the conclusion that as the intake of nicotine increases in a pregnant woman, chances of a delayed birth or physical deformities increases. After giving our zebrafish 3 different concentrations of nicotine, we observed an increase of nicotine led to increased physical problems. For example, in well D2 that had a nicotine concentration of 0.2 mg/ml, there was a fish with a curved spine, and other hatched fish were unresponsive when water was pushed around them. Also the zebrafish exposed to the highest concentrations of nicotine (.2 mg/ml) had the lowest number of fish hatch after 72 hours. However, there was one source of error; in well D1 we counted the number of eggs wrong on the second day. Instead of there being 9, we said that there were 8. The data we collected demonstrates that nicotine can cause problems in an egg or unborn child. In our experiment, we were surprised by the number of hatched fish in wells D 1-3. However, the hatched fish did have physical deformities and were weak, so nicotine had affected them after hatching. The information we learned through this experiment is relevant to human health because it proved how smoking is an extremely large cause of birth defects, especially to the nervous and skeletal systems as evident by the high rates of curved spines and lack of response to stimuli in the highest nicotine concentration group.

Further Question:

If we had exposed the zebrafish to 1.5 mg/ml of nicotine, how would this affect the zebrafish development? Would the eggs be too weak to hatch? Would they hatch but the spine would be crooked?

Works Cited

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