Nicotine and Zebrafish Embryos Life Span

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Abstract

This experiment was done to show how nicotine can affect the human body and early fetal development. The experiment data and observations show that if nicotine is present during a pregnancy, the baby has a high risk of birth defects and deformities because nicotine is a very negatively effective drug. Zebrafish embryos were chosen to represent the human fetus because the zebrafish nervous system and embryonic structure are closely related to human fetuses. For the experiment to be done correctly, a 3x4 well plate was needed and in each well a different concentration of nicotine.The four wells contained nicotine concentrations of 0.00 mg/mL (control), 0.05 mg/mL, 0.10 mg/mL and 0.20 mg/mL. The zebrafish were monitored closely through a microscope and the data was collected daily to show how many zebrafish embryos died or hatched, and when. The data showed that any concentration of nicotine higher than 0.05 mg/mL can put the zebrafish embryos at risk by harming the embryos and shortening the life spans. Any harm that was done to the zebrafish embryos were death and slower hatching rates. Connecting to human health, nicotine can cause similar harmful effects to the human fetus causing the baby to be born too late or early or to be miscarried.

Introduction

Approximately 12-22% of the population, or one million women, in the United States smoke while pregnant (Subbarao, 2006). One of the main reasons for this is the addictive drug nicotine. Nicotine is proven to have many harmful effects on the vertebrate nervous system. Nicotine is a very known and addictive chemical found in many sources including, but not limited to, cigarettes, vapes and cigars. During pregnancy, smoking can result in a wide variety of cognitive, achievement and behavioral disorders, such as ADHD that have been found in the children of the many women who smoke during pregnancy (Van Meurs, 1999). The nicotine, carbon monoxide, and numerous other poisons you inhale from a cigarette are carried through your bloodstream and go directly to your baby (WebMD, 2019). Smoking can not only reduce milk production, but some of the chemicals in cigarettes can pass from the mother to the baby through breastmilk (Better Health, 2019). Facts say that babies and children exposed to secondhand smoke may also develop asthma, allergies, more frequent lung and ear infections, and are at a higher risk for sudden infant death syndrome (SIDS) (WebMD, 2019). Everytime a pregnant woman smokes a cigarette, it cuts down oxygen to her unborn baby and exposes the baby to a cocktail of chemicals, including chemicals that cause cancer (Better Health, 2019). Numerous studies have investigated the incidence of childhood cancer in the children of women who smoked during pregnancy (Van Meurs, 1999). Provenly, the presence of cotinine, a nicotine metabolite with a long half-life, was independently and significantly related to an increased risk of miscarriages (Van Meurs, 1999). Many reports say that a woman who smokes while pregnant is at an increased risk of a wide range of problems including ectopic pregnancy, miscarriage and premature labor (Better Health, 2019). Due to the overwhelming information and questions regarding the effects of nicotine on developing fetuses, research is continuously being done. An experiment containing zebrafish can help answer the question on how much nicotine a fetus or a developing embryo can be subjected to and survive, how various amounts of nicotine can result in deformities and disorders, the effects of nicotine overall on embryos. Zebrafish embryos were

chosen for this experiment because zebrafish have similar comparative embryonic development to humans and it would be very simple to experiment and be able to receive accurate results at the same time. The hypothesis behind the experiment is that zebrafish embryos exposed to the highest concentrations of nicotine will have a lower chance of survival and a lower hatching rate than those that are exposed to lower concentrations.

Materials and Methods

Materials

- 3 bottles of stock solutions of nicotine (0.05mg/mL, 0.1mg/mL, 0.2mg/mL)
- 1 beaker for dead embryos and liquid disposal
- 1 sharpie
- 1 bottle of instant ocean/embryo media solution
- 2-3 transfer pipette
- 1 multi-well plate (3x4)
- 1 28.5*C incubator
- 1 glass slide
- 1 compound light microscope
- 1 stereoscopic / dissecting microscope
- 20 zebrafish embryos
- 1 camera
- 1 petri dish

Methods

On day one, collect twenty rinsed zebrafish embryos and place them into a petri dish. Obtain a 3x4 well plate and label the well plate by using a piece of masking tape. Place the masking tape on the well plate lid above the top row. The control well (0.00mg/mL) will be on the left of the well plate. The three experimental wells (0.05mg/mL, 0.1mg/mL, 0.2mg/mL) will be on the right of the the control well and should be labeled from left to right respectively, beginning just right of the control well. The label should contain the nicotine concentrations of the experimental and control wells. Next, fill the first well on the upper most left side with 1 mL of Instant Ocean solution using a wide pipette. Then, fill the experimental wells (to the right of the control well) with 1 mL of the corresponding nicotine solutions indicated on the label. Be sure to put the correct solutions in their proper wells. Using a narrow pipette, place approximately five embryos in each well containing solutions Record the exact numbers of live embryos on the data sheet. Make sure to observe the embryos under the microscope, and to record any observations on the data sheet. Finally, place the well plate in the 28.5*C incubator and leave overnight.

On day two, three, and four, remove the plate from the incubator. Once in the lab space,remove the dead embryos from the plate using the narrow pipette. Squirt the dead embryos into the waste beaker. Dead embryos normally look white to the naked eye and black and fuzzy on the inside while under a microscope. Be careful to only remove the dead embryos or the data will be inaccurate. Next, count the remaining embryos and any of the hatched fish and record in the data table. When all data is recorded, freshen the solutions from the well plate by removing the old

solutions and replacing them *(Note: Tilt the plate so the embryos settle to the bottom and remove the liquid from the top of the plate).* When replacing the solutions with fresh solution, use a clean pipette. A clean pipette should be used for each of the solutions to avoid cross contamination. Observe a single embryo by placing the well-plate under the microscope and record observations. Finally, return the plate to the incubator.

On day five, remove the plate from the incubator. Once in the lab space, remove the dead embryos from the plate using the narrow pipette. Squirt the dead embryos into the waste beaker. Dead embryos normally look white to the naked eye and black and fuzzy on the inside while under a microscope. Be careful to only remove the dead embryos or the data will be inaccurate. Next, count the remaining embryos and any of the hatched fish and record in the data table. When all data is recorded, observe a single embryo by placing the well-plate under the microscope and record observations. Finally, place all the embryos and fish in the waste container and properly dispose of them.

Results

Due to the overwhelming information and questions regarding the effects of nicotine on developing fetuses, research is continuously being done. An experiment containing zebrafish can help answer the question on how much nicotine a fetus or a developing embryo can be subjected to and survive, as well as how various amounts of nicotine can result in deformities and disorders, in conclusion the effects of nicotine overall on embryos. The hypothesis behind the experiment was that zebrafish embryos exposed to the highest concentrations of nicotine will have a lower chance of survival and a lower hatching rate than those that are exposed to lower concentrations. For this experiment there are three variables, the control, the independent and the dependent. The independent variables were the nicotine concentrations, the dependent variables were the zebrafish life spans and hatch rates, and the control variables were the well plates and the number of zebrafish to begin with. The experiment results related to the death of zebrafish embryos and human fetuses by demonstrating that the higher concentrations of nicotine had higher risks of negative effects. The only zebrafish embryos that died in the control well were due to high unintentional temperatures. Despite that, following deaths in the control well did not occur. Only one seizure was present, and it was in the third well (0.10 mg/mL). In the fourth well (0.20 mg/mL), all zebrafish embryos died by day four, due to the highest concentration of nicotine. The death both in the second well (0.05 mg/mL) and the third well (0.10 mg/mL) did not increase in high numbers by day four, both well-plates had very similar death results. The hatching rate was not similar between the first two wells and the third well. Any death results can be proven in table one.

Table One:

Number of Embryos **Alive vs Dead (Alive/Dead)** at Different Concentrations of Nicotine over One Week

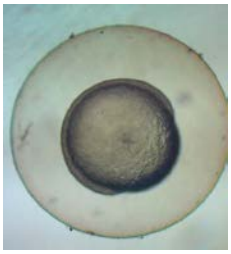
Time	hpf	Control Group (well #1)	Experimental Group Concentration 0.05 mg/mL	Experimental Group Concentration 0.10 mg/mL	Experimental Group Concentration 0.20 mg/mL

		Alive / Dead	(well #2) Alive / Dead	(well #3) Alive / Dead	(well #4) Alive / Dead
Day 1	0 hpf	5/0	5/0	5/0	5/0
Day 2	24 hpf	3/2	4/1	4/1	3/2
Day 3	48 hpf	3/0	4/0	3/1	1/2
Day 4	72 hpf	3/0	3/1	3/0	0/1
Day 5	95 hpf	3/0	3/0	3/0	0/0

Table Two:

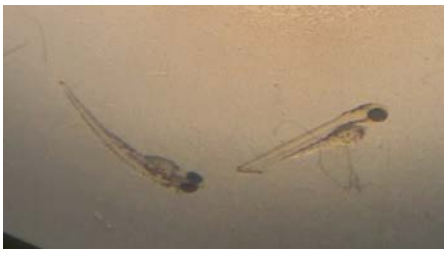
Number of Embryos **Hatched vs Unhatched (H / U)** at Different Concentrations of Nicotine over One Week

Time	hpf	Control Group (well #1) H / U	Experimental Group Concentration 0.05 mg/mL (well #2) H / U	Experimental Group Concentration 0.10 mg/mL (well #3) H / U	Experimental Group Concentration 0.20 mg/mL (well #4) H / U
Day 1	0 hpf	0/5	0/5	0/5	0/5
Day 2	24 hpf	0/5	0/5	0/5	0/5
Day 3	48 hpf	0/3	0/4	0/3	0/2
Day 4	76 hpf	3/0	1/2	0/3	0/0
Day 5	96 hpf	3/0	1/2	0/3	0/0

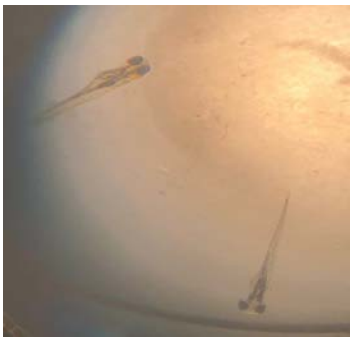


All Well-Plates, Day 1

Well-Plate 1, Day 2



Well-Plate 1, Day 4

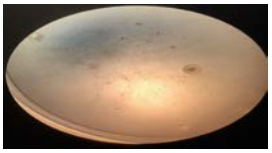


Well-Plate 1, Day 5

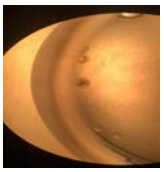
Well-Plate 2, Day 2

Well-Plate 2, Day 4

Well-Plate 2, Day 5

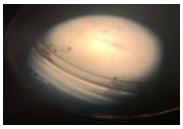


Well-Plate 4, Day 2



Well-Plate 3, Day 2

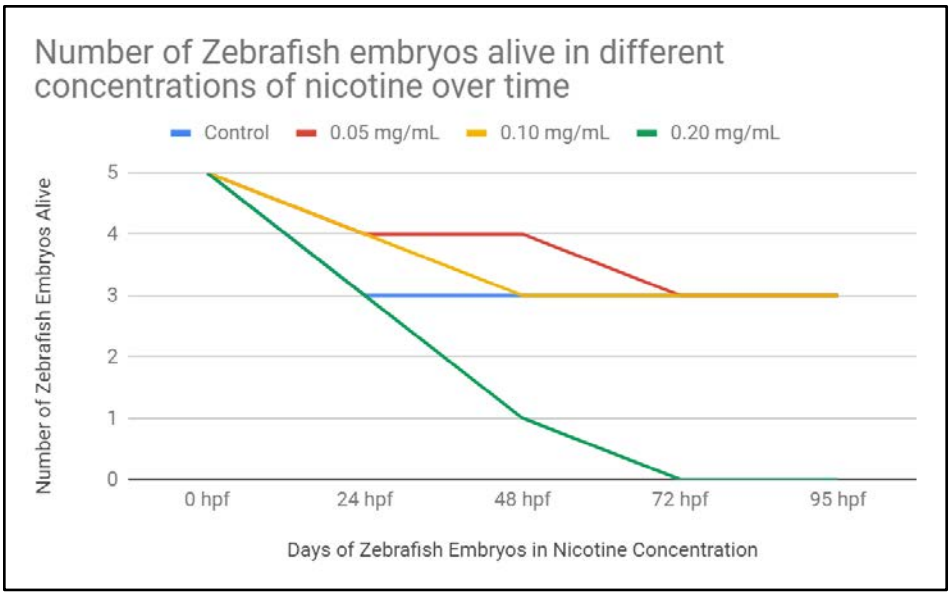
Well-Plate 3, Day 4



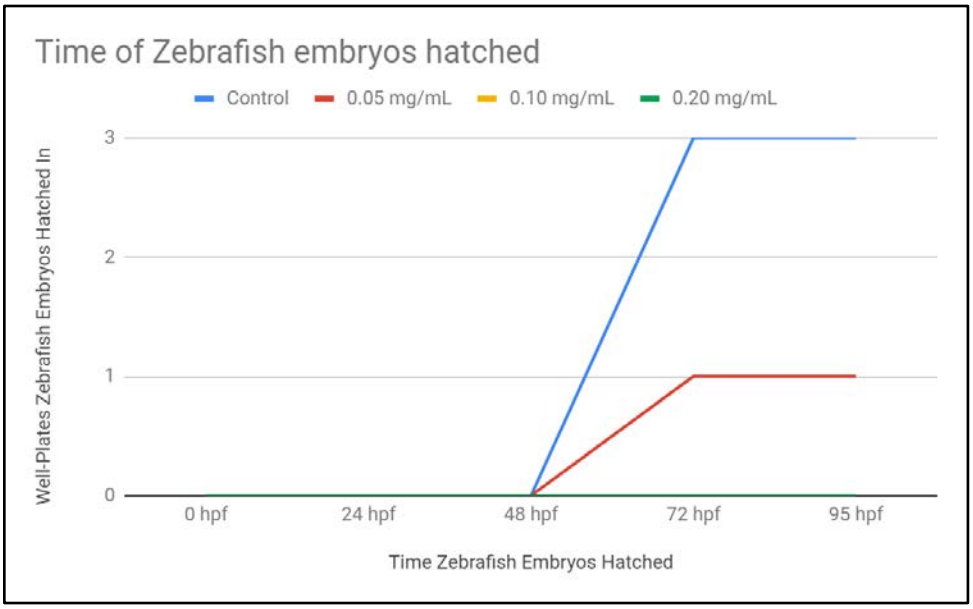
Well-Pate 3, Day 5

Well-Plate 4, Day 4

Graph One:



Graph Two:



Discussion

After five days of documenting the zebrafish progress, it was proven that the hypothesis that high levels of nicotine have a negative effect was correct. The experiment results evidently support the hypothesis with photo evidence and data. Zebrafish embryos were chosen for this experiment because zebrafish embryos have similar comparative embryonic development to human fetuses and it would be very simple to experiment and be able to receive accurate results at the same time. On day one, zebrafish embryos were obtained, but due to accidental rise in a temperature of 30°C in the incubator over the night, one to two zebrafish embryos died in each well-plate. All of

the following information can be viewed in graphs one and two. On day two after dispensing 0.00 mg/mL in the control well, two of five zebrafish embryos that were received were found dead and zero hatched. In the second well containing 0.05 mg/mL of nicotine concentration, only one out of the five zebrafish embryos were found deceased, with zero embryos hatched. After adding 0.10 mg/mL of nicotine concentration in the third well, zero embryos were hatched and only one of the five zebrafish embryos were found dead. Lastly on day two in the fourth well containing 0.20 mg/mL of nicotine concentration, two of the five zebrafish embryos had died, and zero embryos were unhatched. On day three, in the control well zero zebrafish embryos had died nor hatched. In the well containing 0.05 mg/mL of nicotine concentration, zero zebrafish embryos had died nor hatched. In the well containing 0.10 mg/mL of nicotine concentration, zero zebrafish embryos had hatched but one had died. In the last well of 0.20 mg/mL nicotine concentration, two zebrafish embryos had been found deceased and zero hatched. On the fourth day in the control well, zero zebrafish had died and all had hatched, remaining the same for day five. In the second well of 0.05 mg/mL of nicotine concentration, zero zebrafish embryos had died, and only one had hatched and this data remained the same for day five. In the third well of 0.10 mg/mL nicotine concentration, zero zebrafish embryos had died nor hatched and this remained the same for day five. In the last well of 0.20 mg/mL nicotine concentration, only one zebrafish embryo was found and it was deceased and unhatched, there was no data to be collected on day five due to zero zebrafish embryos. Any trends found in the data were that the higher amount of nicotine concentration, the higher amount of deaths and the smaller amount of hatched embryos. Even further into the experiment the results show that the hypothesis can in fact be proven by the data. Towards the end of the of the experiment it showed that nicotine affects the body in a highly negative way. For example, some negative effects nicotine can cause are cancer (Better Health, 2019) and ADHD in the children of the women who smoke during pregnancy (Van Meurs, 1999). In the well of the highest concentration of nicotine resulted in zero zebrafish embryos alive by day four. In the control well despite the two dead embryos on day one due to temperature, no other zebrafish embryos had been found dead and all were hatched by day four. An additional example is that in the 0.10 mg/mL concentration of nicotine it had one zebrafish that was having a seizure every few minutes. Possible limitations of the experiment included the temperature of the room that the embryos were stored in a room too warm it was approximately 30°C (86°F). Another limitation was that on day three it was not possible to obtain pictures of the zebrafish which then in turn limited the data that could have been collected. Any improvements that should be made for this experiment include being able to check the temperature and having it be at an appropriate temperature before allowing the zebrafish embryos to be left overnight. In conclusion, the results supported that the hypothesis could be supported through the data from the experiment and furthermore through additional research. The results of this experiment were very accurate and inserted knowledge for any research that any concentration of nicotine can be very harmful towards the human body and human fetuses, but precisely the highest concentrations are the most harmful.

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