Effects of Nicotine on the Development of Zebrafish Embryos

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March 10th, 2017

Abstract

The purpose of conducting this experiment was to confirm previously collected data and observations as well as any and all related development abnormalities. In order to confirm or deny any data collected in the past, Zebrafish embryos were placed in different environments with varying levels of nicotine concentrations. The embryos were exposed to this environment from fertilization and held until five days past. As the embryos were observed every 24 hours their spinal deformities became more apparent the farther from fertilization, markedly the higher the concentration of nicotine in their environment. These results are similar to the effects on human embryos when nicotine is present during pregnancy. These results support some of the findings of past researchers in this area.

Introduction

Much research has been done in this area. The research tells us that when the stimulant, nicotine is present in an embryo's environment - usually because of smoking - the embryo's development is delayed; sometimes so much so that the organism dies. Previous research also tells us that when nicotine is present at birth and or during pregnancy, the organism may be born premature or underweight, may have Sudden Death Syndrome, cardiovascular defects, a cleft lip and palate, immunodeficiency(2), and cognitive, intellectual, and behavioral deficiencies(1). In turn, a Zebrafish embryo exposed to nicotine may hatch early, are less likely to hatch, may be of smaller size, have cardiovascular defects, and spinal deformities.

After completing the experiment, more research could be done to see what effects specific concentrations of nicotine present at different points of an embryo's development would have on the embryos development.

At the beginning of the experiment nicotine will be introduced into the surrounding environment of Zebrafish embryos. The effects on the embryos and the newly hatched fish will be observed and recorded. A comparison will then be made between the number of living fish, the development, and the homeostasis to other, non-drugged embryos.

In four wells of the well plate an embryo will be placed with 0, .05, .1, and finally .2 mg/mL of nicotine respectively. At 0 hours, 24, 48, and 96, each well will be emptied and observed under a microscope. During the observation time, the number of fish hatched, number living, and the development (appearance and behavior) of each fish will be recorded. After the remaining fish have been euthanised the recorded data and any other observations will be put into graphs and data tables, compared to results from similar experiments using other drugs, and will also be used to predict what will happen to a human fetus if nicotine is present at birth.

The results of the experiment are expected to repeat, confirm, the data collected from previous research and experiments. The the more nicotine present in the embryo's environment, the less will hatch, the more will hatch early, the less will live the 96 hours of the experiment, and more of the living fish will have defects.

Materials and Methods

Materials

- Embryo Media/Instant Ocean
- One bottle of 0.05 mg./mL. concentration of nicotine
- One bottle of 0.1 mg./mL. concentration of nicotine
- One bottle of 0.2 mg./mL. concentration of nicotine
- A container for dead embryos and waste solution
- Minimum bore 1.5mm. pipettes
- 1mL. pipettes
- One well plate

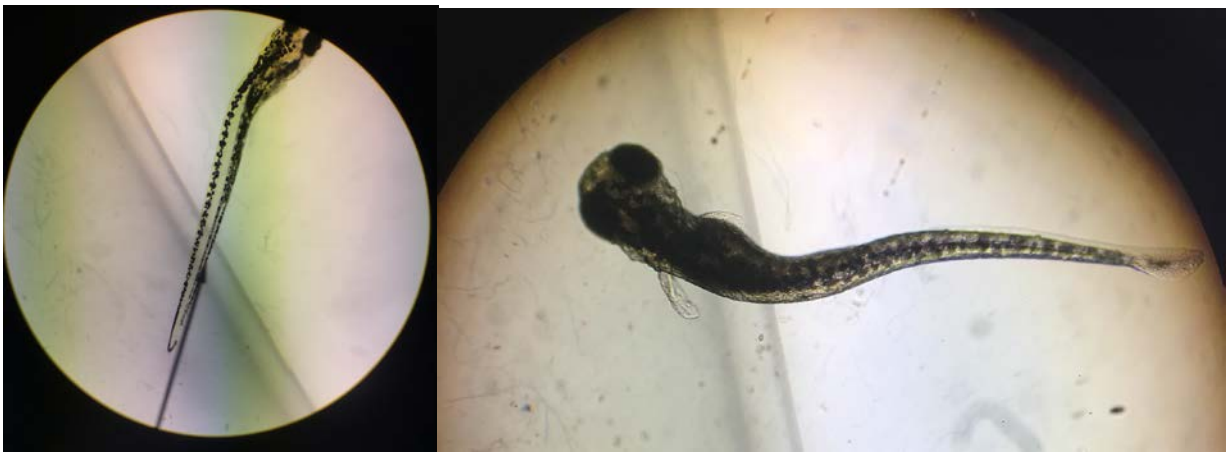
- One incubator set at 26°C to 28°C
- One depression slide
- One dissecting microscope
- One compound microscope
- Zebrafish embryos
- One camera - a smartphone camera works very well (optional)
- Data and observation sheets
- A writing utensil
- Three strips of a solid color tape and a permanent marker (optional to label on the outside, the wells, and or which well plate is yours)

Methods

The first step was to fill one of the wells in the well plate with 1mL. of Embryo Media, the next with 1mL. of .05 concentration of nicotine, the third with 1mL. of .1 concentration of nicotine, and finally the fourth with 1mL. of .2 concentration of nicotine. Ten live embryos were placed into each well by a minimum bore pipette. While placing the embryos in the wells discard of any dead by releasing them into the waste container. After all the embryos are in their places, the live number(10) for each well was recorded in the data chart. The dissection microscope was used to examine the embryos in the wells, while depression slides and a compound microscope was used to examine them individually. Observations were made every 24 hours and added to the data sheet as well as pictures of an embryo from each well as an example of and easily seen progression, or lack of progression, in development from day to day. When all observations were made and recorded and the embryos were returned to their respective wells, and the covered well plate was put into an incubator; its temperature ranging from 26° to 28°C. On following days (days two, three, and four) the well plate was brought out of the incubator, the dead embryos removed and the number recorded, live embryos as well as those that had hatched recorded, and the solution in each well disposed of into the waste container. Fresh Embryo Media and nicotine concentrations filled their resective wells. Then, observations were made and recorded and pictures were taken of an embryo from each well with the compound microscope and depression slides. After the observations were complete, lid was replaced on the plate, and the plate was returned to the incubator. On day five the last observations were recorded and both the solutions and the embryos were discarded into the waste container. At the end of day five the embryos were euthanized.

Results

This experiment was conducted to confirm and repeat data collected from previous research. The prediction was that the more nicotine was present in the embryo's environment, the less will hatch, the more will hatch early, the less will live the 96 hours of the experiment, and more of the living fish will have defects and or deformities. The four wells used during the experiment held ten Zebrafish each. The first was the control and contained no nicotine while the remaining three progressively contained more (.05, .1, and .2 mg./mL.). The concentration of nicotine was the independent variable and the dependent variable was the number of remaining, live embryos and the level of their development. While the embryos hatched at about the same time, each well had about the same number of embryos throughout the experiment, and all of the fish were about the same size; there was a clear, severe deformity in the embryos spines that also had nicotine present in their environment. The more concentration of nicotine in their environment, the more severe the deformity. These results support pieces of the hypothesis. And while the Zebrafish prove excellent test subjects, they can not be tested for cognitive, intellectual, and behavioral deficiencies that are found in humans when they were exposed to nicotine at birth.



Number of Living Embryos

CONCENTRATION OF NICOTINE	WELL NUMBER	DAY 1 0 HOURS	DAY 2 24 HOURS	DAY 3 48 HOURS	DAY 4 72 HOURS	DAY 5 96 HOURS
Control	A1	10	10	8	8	8
.05 mg./mL.	A2	10	9	9	9	9
.1 mg./mL.	A3	10	10	9	9	9
.2 mg./mL.	A4	10	10	10	1	10

Number of Hatched Embryos

CONCENTRATION OF NICOTINE	WELL NUMBER	DAY 1 0 HOURS	DAY 2 24 HOURS	DAY 3 48 HOURS	DAY 4 72 HOURS	DAY 5 96 HOURS
Control	A1	0	0	0	0	8
.05 mg./mL.	A2	0	0	0	1	9
.1 mg./mL.	A3	0	0	0	2	8
.2 mg./mL.	A4	0	0	0	0	9

Discussion

The spinal and deformity was the most severe difference between the control embryos and those exposed to nicotine. This difference is most likely related to nicotine being a stimulant, and its toxins increasing the activity of a gene that causes stem cells to be nearly duplicates of each other(1), causing mutations that result in deformities. Spinal deformities appear to be the only result similar to those found in other experiments.

Many things could have happened, intended or not, that would have changed the results of this experiment. For example, mistakes could have been made putting fresh Embryo Media and or the nicotine concentrations into their assigned wells, replacing an embryo into the proper well after examination, unwanted materials or solutions entering the wells used in the experiment, or missing counting the number of living fish or the number of those hatched. These mistakes are unlikely however, extreme care was used in removing and replacing old concentrations, replacing the fish into their wells

unharmed, keeping other liquid and materials, such as food, away from lab stations, and before any numbers were permanently written into data tables, they were double and triple checked.

While the results do repeat and confirm the data collected from previous researcher's experiments, such as the spinal deformities, it does not confirm smaller size, less hatched, or more hatched early, as the concentration of nicotine increases.

Even if this experiment confirmed and repeated all results come across by other researchers, more research should be done to see if and what different concentrations of nicotine released into the environment at different points of the embryo's development would result in regarding the embryo's development. Research could also be done to see if anything would lessen the effects of Nicotine on the development of the embryos with the same concentrations in the environment.

References

(1) Kurt R. Svoboda, Sukumar Vijayaraghavan and Robert L. Tanguay (2002). Nicotinic Receptors Mediate Changes in Spinal Motoneuron Development and Axonal Pathfinding in Embryonic Zebrafish Exposed to . *The Journal of Neuroscience.*

(2) Jennifer O'Brien (2012). Tobacco Smoke Affects Early Human Embryonic Development. *University of California San Francisco.*