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Ethanol Impedes Heart Rate And Body length In Chick Embryos

Hemant Patel

Last Updated: 12/02/04

I. Introduction

The Chicken embryo, (taxon Gallus gallus) develops and hatches in 20-21 days and has been extensively used in embryology studies especially in Developmental Biology. Their availability and similarities with mammalian embryo help shape our present understanding of embryology. Fertilized eggs can be easily maintained in humidified incubators and the embryo floats on to the egg yolk that it is using for nutrition, which can be easily viewed in any compound microscope (2004, Hill). Chick embryos in a proper state of preservation and the stages desired can readily be secured and prepared for study. For many researchers and scientists they offer a basis for understanding the early differentiation of the organ systems and the fundamental processes of body formation common to all groups of vertebrates. In fact the chick embryo serves as a type of developmental characteristic of large-yolked eggs of birds and reptiles, and as an intermediate form bridging the gap between the simpler processes of development in fishes and amphibian (Patten 1927).

Many experiments can be carried out using chick embryos using variable testing factors, such that the information or the data that is gathered can help answer many of the unanswered questions in wide array of Developmental Biology. The results that we obtain from testing these different factors can be applied to other mammals, even humans in some instances that would lead to beneficial outcomes.

One of the popular experiment that is well known is the Fetal Alcohol Syndrome (FAS), which is caused by exposure of the developing embryo to alcohol, one of several teratogenic agents which adversely affect the developing embryo (2004, Zagory). Studying the FAS in chick embryos and how it ends up affecting many of the developing organs in the embryo will help us guide to some of the answers that we can relate to FAS in humans, as more and more cases relating to fetal alcohol syndrome are being reported now a days than they ever were.

FAS is one of the most common birth defects in Western World, specifically characterized by the growth and mental retardation, craniofacial malformations and heart and neural defects. It is expected that one baby out of 500 to 3000 annual live births will have FAS (Unnamed, 2002). Although FAS is found to be affecting almost all of the new developing organs it serves and has been one of the major studied topics in biology. Ethanol, better known as alcohol, is an organic compound that is linked to having teratogenic effects on almost all of the developing organs in a human fetus. Ethanol effects have also been studied among other organisms, and these effects primarily depend on the concentration of the ethanol, as well as the time period for which it is exposed. Experiments with ethanol and its

teratogenic effects on the human fetuses are merely impossible due to the ethical questions that they raise and the complexity that is involved. However, what scientists have learned to adapt throughout history, is to work with something that is simple and testable, and apply that to something that is a little bit more complex (Patel, 2004).

With this in mind, one can easily experiment with ethanol and its teratogenic effects on developing chick embryos, a popular research topic among developmental biologists. One experiment can be designed that would test the effect of ethanol on the heart rate of the developing embryo, and how that correlates to the corresponding body length of that embryo. Since the heart pumps blood, throughout the body, and the heart rate determines the how efficient that the heart is working, one would hypothesize that embryos that are injected with alcohol concentration would develop a higher heart rate due to the alternation in the lipid concentration caused by ethanol (Ruiz-Gutierrez, 2004). This contracts the heart more than it should, so therefore embryos that are found in the high level concentration of ethanol will have the highest heart rates. At the same time body length of the embryo will also be monitored in ethanol where alcohol induced embryos will have retardation of body length similar results to what Yang obtained when experimenting FAS with Xenopus embryos (Yang, 2004). So therefore embryos induced in high concentrations of ethanol will have a higher percent of body length retardartion.

II. Materials and Methods

Materials:

The materials that were used during this experiment were the following: BVT-200 pro software, Microsoft Excel software, Sony DFWX700 camera, compound microscope, kem-wipes, paper-towels, petri-dishes, weigh boats, paper towels, 70% sterile ethanol, concentrated ethanol, Tyrode's solution, fertilized eggs, sterile pipet, syringe, forceps, micropipette, ruler and incubator.

Procedure & Methods:

We obtained 48 fertilized eggs, directly from the shipment companies that were placed in the incubator for 2 days. On third day all 48 of them were distributed among the 6 groups so each group received 8 two day fertilized eggs. Each group had to use the same procedure for the lab, but however each group will be testing different factor(s) that will be affected by Fetal Alcohol Syndrome (FTS). However, the groups were to collect some similar data, that had to be share among the class such as heart rate, vascular area, body length, allantois, wingbud, and limbbud which had to be observed and data had to be recorded everyday for each of the embryo that were explanted and stayed alive. So therefore the data sample collected were from samples from all of the six groups. The factor that I will be testing was the heart rate and body length, in which the chick embryos that are injected with two different concentrations of 0.2% and 2.0% ethanol, (the experiment groups) compared to the chick embryos that are NOT injected with ethanol, serving as the control group. This experiment was one week long, therefore we conducted only two trails.

The 4 control group embryos as well the 2 each of the different ethanol concentrations embryos were monitored by altering member from our group, for the seven consecutive days, to gather all the necessary data for our group as well as the class data. The body length, one of my focus topics, included data that was obtained through measurements from similar embryos using mm ruler as a guide, and ocular prediction to be as accurate as possible. Similarly my other focus topic, the heart rate of the each alive embryo will be observed through the use of Sony DFW700 camera hooked up to a compound microscope, which is supported by BVT 200 software for Macintosh. The observations for this experiment did not take more than few minutes for each embryo, since you were only observing the heart rates according to the time and measuring the body length. The heart rate data was quantified using beats per minute (bpm) as units from day three to day six. Similarly, the body length was quantified by measuring the embryos relative size using millimeters (mm) as units that corresponded to different days. These data that were collected, included our group's data, as well as the class data through observations made on the embryos for seven consecutive days for each of the two weeks that corresponded to the two trials. The class data came from the other groups who made similar observations, for two weeks. All groups data were entered into one Microsoft Excel spreadsheet and this spreadsheet was available from Bio254 folder, on icuc web server.

To make our results more accurate class averages were taken rather than just analyzing our own groups data, by

taking the mean for the measurements that corresponded to heart rate and body length for each of the days. Another reason to quantify my results using averages was, to produce a better overall trend for both the body length data as well as the heart rate. Although there were total of 96 embryos that were used by total of six groups in the two trials, with the mortality rate of the embryos being high, the data results were minimum even after taking averages. Once the averages were obtained from using the class data, I decided to analyze my data using Microsoft excel spreadsheet software, to create two graphs. One graph would compare the average body length to its relative day for each of the control and experimental groups and similarly the other graph would compare the average heart rate to its corresponding day for each of the groups. These would help study and visualize the different trends associated with each of the groups.

Fertilized eggs were first incubated for 2 days at 37.0 C and than were ready to use for our experiments. The eggs after incubation were sterilized using 70% ethanol, to ensure that there is no contamination. To take better pre-cautions all of the materials that will came in contact with the egg or the embryo were sterilized with ethanol, including the lab bench where explanting of the eggs took place. Kim-wipes were used to fully dry out the weigh-boats and petri-dishes that were sterilized with ethanol. Paper-towels were used to clean lab bench, after sterilizing it with ethanol. Total of 8 petri-dishes and weigh-boats were obtained and all of them were sterilized with ethanol. Then the weigh boats were slightly bended from each of the four corners, in order to prevent seal ups when they were placed into petri-dishes. The eggs were than explanted into the petri-dishes using either one of two techniques. Our group simply cracked opened the egg on the bench and slowly poured out the embryo in the weigh boat. Other technique that some groups used was to take forceps and create a sufficient hole through which the embryo was poured out by making another hole from the other side of the egg.

After explanting each of the eight eggs into the weigh-boats, they were placed into petri-dishes and covered to minimize the chances of contamination. However, during explanting we accidentally destroyed one of the embryos, which had to be discarded out. Then we labeled all of petri-dishes using Sharpie marker with 3 of them being the control group, 2 ethanol low (0.2%) and 2 ethanol high (2%). Than in each of our control embryos that we explanted, we added 5 ml of Tyrode's solution made in right concentration that was obtained from Professor Morris. Than for our experiment groups, we added 0.5 ml of 2% ethanol (EtOH) in 5 ml Tyrode's solution that was calculated with the help of Professor Morris, in two embryos labeled high ethanol. Then in a similar manner we had to add 0.05 ml of 0.2% EtOH in 5 ml Tyrode's solution for the remaining two low ethanol embryos, once again wit with the help of Professor Morris. After adding the Tyrode's solution, we simply added penicillin, in a concentration of 100ug/ml from a stock solution prepared by Professor Morris, in all embryos to prevent bacterial contamination. Then with the lids being closed of the Petri-dishes the embryos were carried out to a different room to incubate them at 37°C and each of the group member was assigned to come in on certain days for the next seven days to observe on all of the embryos.

During the observation, the group member had to the record the following data for each of the embryo that was alive: vascular area, body length, limb bud, wing bud, alantois width, and heart rate. After the first week of data gathering, they were analyzed during the second week. The following week the same exact procedure was followed, in terms of sterile technique and explanting, however the only change was that, we had destroyed two eggs, resulting in 4 control groups and only 1 low EtOH and 1 high EtOH, for the trial of the second week. Once again, one of the group member had to come and observe the embryos for seven consecutive days, and also collect data, in regards to the same factors as in last week. Once all the data was obtained for the second week, all of the groups who were experimenting on Fetal Alcohol syndrome had summit their data into a Microsoft Excel worksheet, that was later available to all groups to do further analysis of the topics that they were pursuing (Armstrong, 1994).

III. Results:

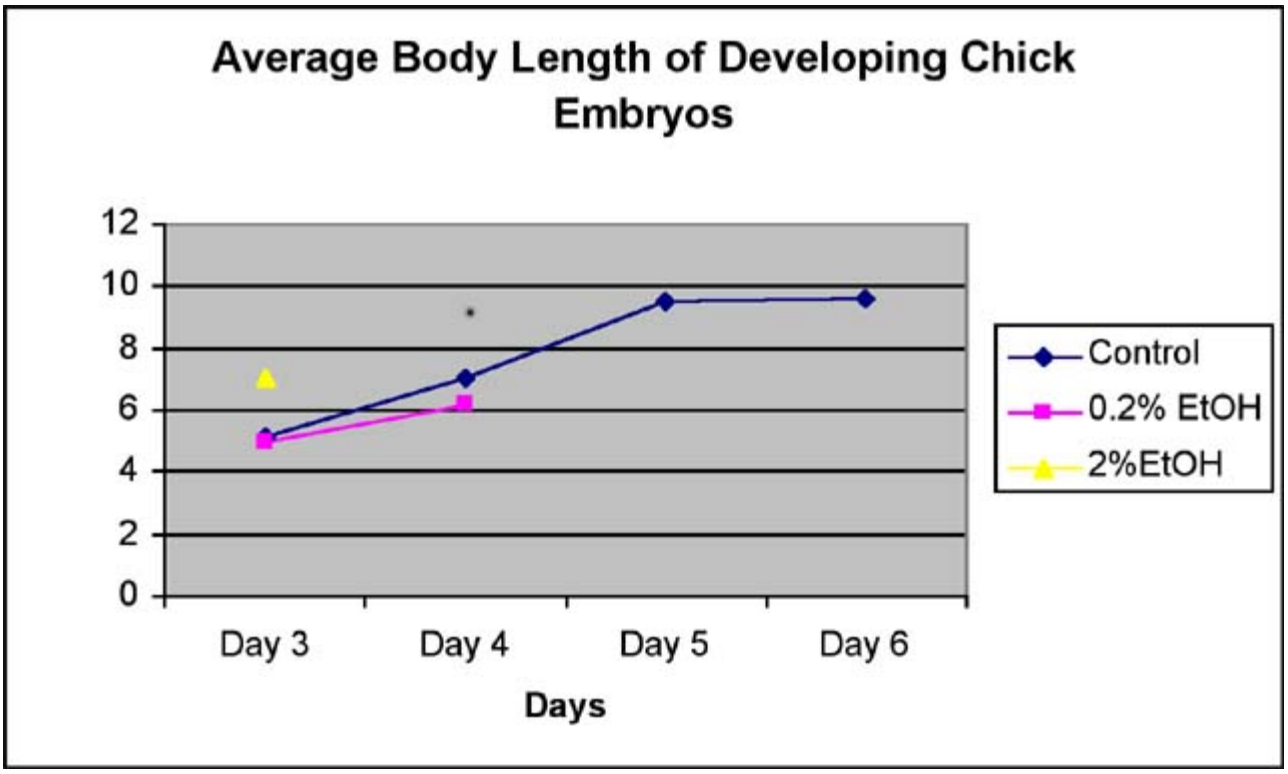

Figure 1—shows a graph that compares average body length of developing chick embryos Over a four day period for the following three groups: control, low EtOH, High EtOH.

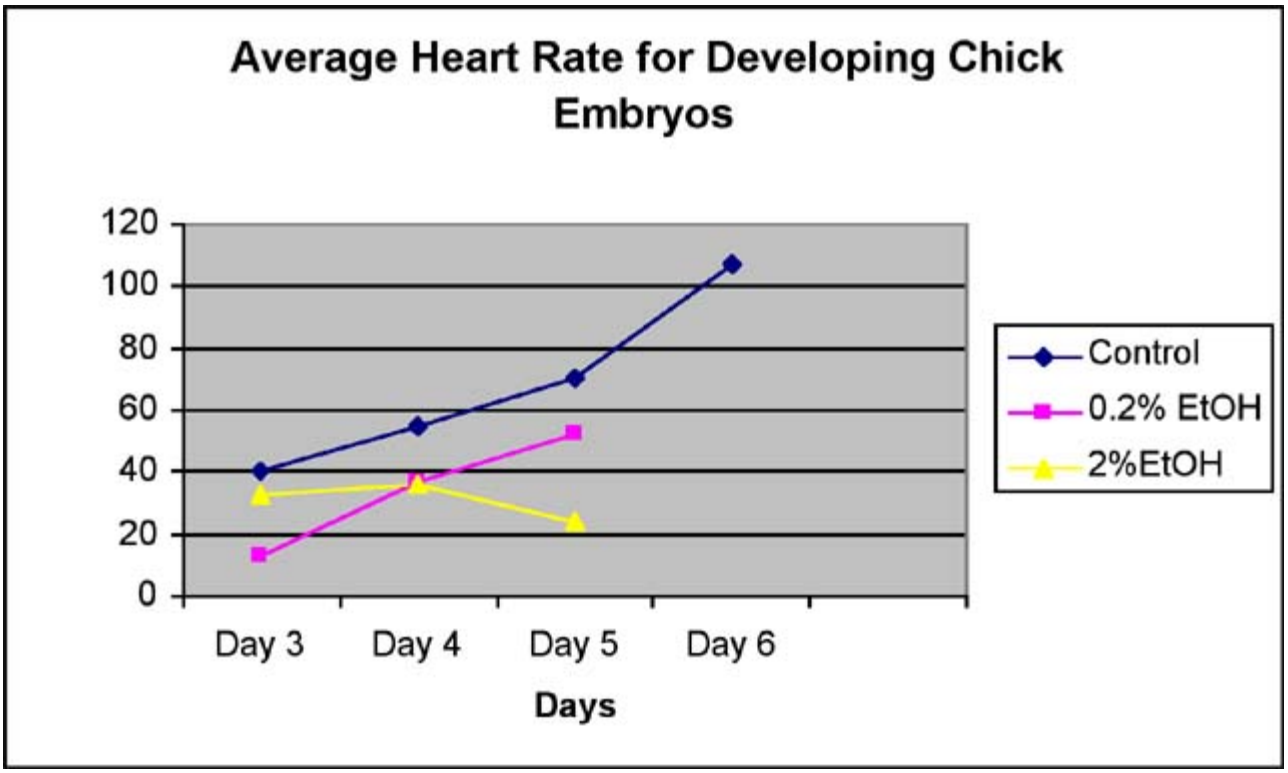

Figure 2—A graph that compares the average heart rate for developing chick embryos in control,
low ethanol, and high ethanol from day 3- day 6.

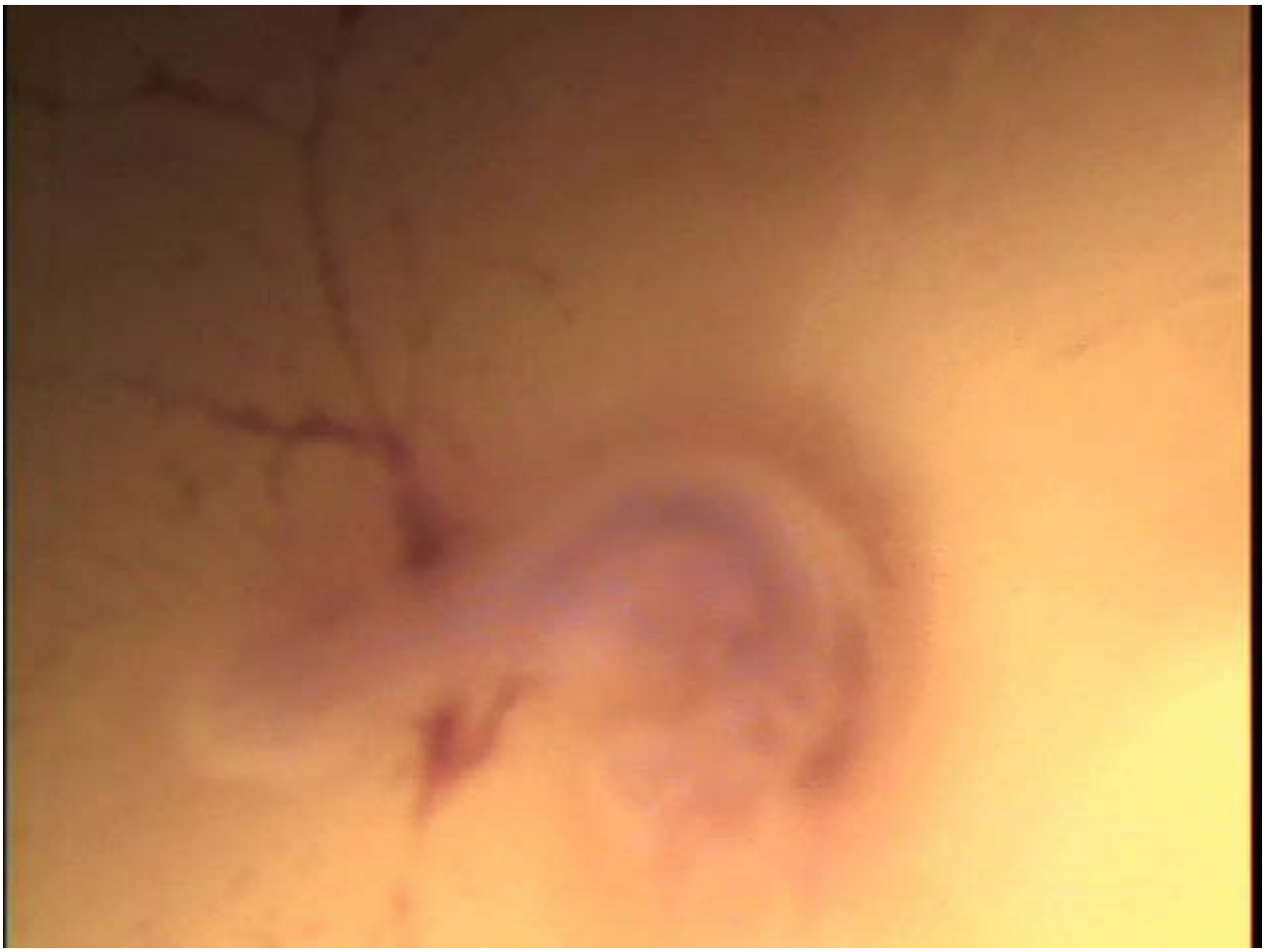

Figure 3--Image that represents the body length of a chick embryo taken during day 4, in low ethanol concentration.

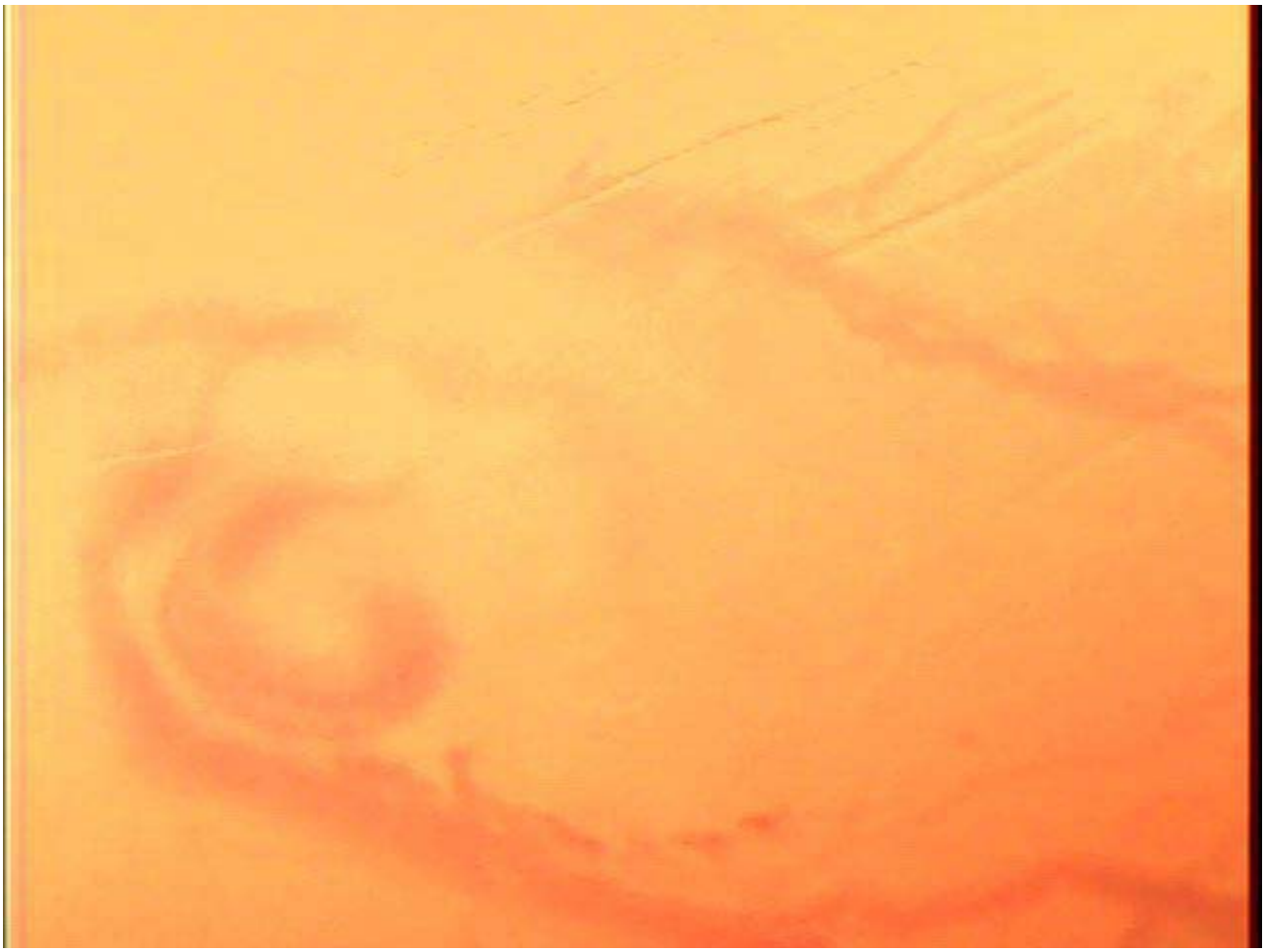

Figure 4--Image that represents the heart (round circle) for a chick embryo during day 3 in low ethanol concentration.

The results were based on the data that were gathered from embryos that were planted during week 1 and week 2. The data were collected from seven groups that were testing the Fetal Alcohol Syndrome on developing chick embryos. The groups had collected data from day 3, which corresponds to the first day after explanting through day 6, point where none of the embryos were alive. The results relate to the study that I was conducting, to study the effects of ethanol on the heart rate (where the heart of the chick embryo can be observed in figure 3) and at the same time how that effects the developing body length of the embryo (body length can be observed for the a developing chick embryo in figure 4). Using the class data came out to be very helpful, since we did not have sufficient data, through which any interpretations or conclusions could be drawn. No data was available for day five or day six for the body length that corresponded to the embryos in low ethanol concentration. The same is true for the embryos in high ethanol concentration where no values correspond to either day four, five or six due to the high mortality rate for these embryos.

With this in mind, looking at figure 1, which simply provides a graph that was generated using the averages obtained from class data for the body length each of the control and experiment group from day 3 to day 6. This graph portrays a clear picture by which the trend is increasing and by what factor. As one can see the higher values that correspond to the control group, which can be also seen on the graph, where the line that represents the control group is positioned at a higher corresponding y value. However, the value that represents the body length of chick embryos in low ethanol from day 3 to day 4 are much smaller than the control group and no set of values were available to accurately represent the average body length of the embryos in the high ethanol concentration besides day 3.

Similar trend can be noted in figure 2, graph that corresponds to the values that were obtained from class averages for the heart rate in relation to different days for each of the control and experimental groups. This trend is overall increasing for the heart rate for all embryos in all three groups. One can clearly see that the control group chick embryos have average heart rates that are higher than both the low ethanol and high ethanol inducted embryos. The low ethanol inducted embryos start off with greater heart rate than embryos in the high ethanol concentration, however this

changes during day four, where their average heart rate significantly drops. No heart rate data were available for day six, for both of the ethanol groups due to the high mortality rates after day four.

IV. Discussion and Conclusions

My hypothesis that predicted that embryos developing in the ethanol concentrations will have heart rates that are high compared to the control group, was found to be incorrect according to the results that are obtained through this experiment. However, the heart rate had opposite outcomes, such that the embryos present in the high ethanol solution seemed to have the lowest heart rate, followed by the somewhat higher heart rate by embryos in low ethanol and even higher rate for the embryos in the control group. From this we can conclude, that ethanol does have an impact on a developing heart rate in a manner that leads to repression somehow. This may occur through by ethanol contracting the heart in a manner that lowers its activity. This relates to what Bruyere, found in his experiment where he concluded that reduced embryonic cardiac blood flow occurred when chick embryos were induced with high concentrations of ethanol (Bruyere, 1994).

The second part of my hypothesis that predicted, ethanol would decrease the body length, came out to be correct if interpreted from the graph provided in figure 2. Although we do not really have sufficient data that corresponds the body length of the embryos in high ethanol through which any conclusions can be made, we can certainly predict that the body length of these embryos must be in the range, of the embryos in the low ethanol concentration, since these embryos were facing the same environment with all the g factors were the same.

Similar, predictions and experiments have been conducted by many scientists that ethanol leads to body length retardation and one of them was Yang, who carried out an experiment that tested FAS in Xenopus embryos, and concluded through his results, that concentration of ethanol does lead to retardation of body length (Yang, 2004). Looking at the our overall results, we can conclude that ethanol leads to depression of the heart rate and at the same time leads to body length retardation. From this results we can predict, that the heart due to the lower heart rate will contract less and thus is not able to work as efficiently in supplying the blood thus reducing the blood that is pumped to the other parts of the body, thus reducing the growth which leads to smaller body length.

Similar experiments can be carried out and the results that we obtain, can be made better by looking at the sources or error that we made in this experiment, that we can improve in the future experiments. The biggest problem that we faced in this experiment was the very high mortality rate for the chick embryos. Sources of the error that we made, that might have lead to this, could have been for example leaving the embryo outside for too long, disturbing it optimal temperature or maybe the left the cover of the petri-dish open for too long, during the observations thus leading to contamination. If we can take pre-cautious steps taking some of these sources of error into consideration we can ensure the better survival of the embryos. We can also ensure that enough penicillin concentration is added to prevent contamination, add enough open trays of water to prevent condensation and also modifying the lids so there is proper oxygen exchange, develop a new technique to observe the embryo without opening the lid, or provide a bigger weight boats so there is more surface area for the yolk to rest on (Patel, 2004).

The changes that we make not only apply to chick embryos in future experiments, but to many others birds embryos that are being studied for research purposes. With this in mind we can certainly design future experiments in which we can use greater variation of ethanol concentrations, which would produce a better trend-line that helps us understand the relationship that holds between ethanol and its effects on heart rate and body length. In future experiments we could apply the same procedure, and test it on other mammals embryos and see whether ethanol affects them in the same manner. In future experiments, we could use a greater number of embryos, for our experiments groups, since they had a very high mortality rates so that way we ensure we would have greater number for our results.

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