Chick embryo wing bud length observations in relation to ethanol treatment in a model of applied fetal alcohol syndrome

Rebecca A. Smith
Developmental Biology Short Report
Bio 254 Developmental Biology
Wheaton College, Norton, MA, USA
April 28, 2015

Introduction

Fetal Alcohol Syndrome (FAS) is acquired by the presence of a teratogen on a developing embryo, in this case, alcohol. FAS is one of the most prevalent birth defects in the present Western world. The condition is most commonly characterized by growth and mental retardation, along with cranial abnormalities and heart defects (Zagory *et al*, 2004). Fetal Alcohol Syndrome is completely preventable if the mother abstains from drinking alcohol during pregnancy.

To test the effects of ethanol on embryos, we manipulated chick embryo development with multiple levels of added ethanol. Considering ethanol is a teratogen that induces a variety of developmental abnormalities, this lab complements FAS research ethically. Ethanol when added to nonhuman embryos assesses the time and magnitude of the dose that causes developmental delays (Ahlren *et al*, 2002). This research is important because nonhuman embryos, specifically chick embryos presented with ethanol, can be used to gain an understanding and promote new treatments for FAS in human babies.

Sara Ahlgren, Vijaya Thakur and Marianne Bronnor-Fraser tested the effects of ethanol on chick embryos and observed noticeable developmental delays. Along with abnormal facial structures, the embryos treated with ethanol displayed high patterns of cell death. The dying cells were neural crest cells, normally induced by Sonic Hedgehog (*Shh*). The ethanol presented to the embryos was blocking *Shh*, which in return caused signaling pathways of typical development to be effected. Ahlgren and company observed that ethanol blocks *Shh* signaling, resulting in cranial and other abnormalities. This finding presented a link between FAS and the loss of *Shh*. (Ahlren *et al*, 2002)

In this study, we tested the effects on embryonic exposure to alcohol on chick embryos to test the hypothesis that the presence of ethanol reduces wing bud length during early embryonic development, perhaps by blocking *Shh* signaling.

Methods and Materials

Ten-day chick embryos (Gallus gallus) were explanted from their shells as per the method of Morris (2015) and Armstrong, et al., (1994) except that a sterile weigh boat was used instead of a sterile hammock described by Armstrong. Ethyl alcohol was administered to each embryo to achieve a final concentration on-the-cells of 0.0 % (for control), or 0.002% (for low

ethanol dose), or 0.02% (for high ethanol dose). Penicillin/streptomycin was also added to each embryo to achieve a final concentration on-the-cells of 100 U mg/ml.

Data for control embryo derived from averaging day 3 wing bud length with 13 embryos, day 4 with 13 embryos, day 5 with 13 embryos, day 6 with 9 embryos, day 7 with 8 embryos, day 8 with 7 embryos, day 9 with 5 embryos, and day 10 with 5 embryos.

Data for low concentration embryos derived from averaging day 3 wing bud length with 4 embryos, day 4 with 3 embryos, day 5 with 2 embryos, and day 6 and 7 with 1 embryo. (All low concentration embryos perished after day 7)

Data for high concentration embryos derived from averaging day 3 wing bud length with 14 embryos, day 4 with 13 embryos, day 5 with 13 embryos, day 6 with 11 embryos, day 7 with 10 embryos, day 8 with 9 embryos, day 9 with 9 embryos, and day 10 with 9 embryos.

Results

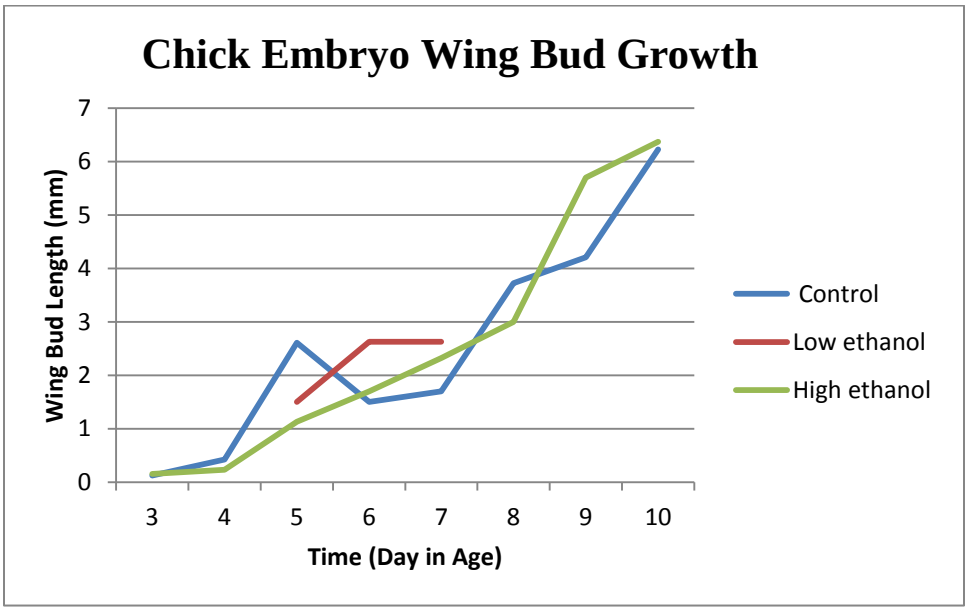

Figure1: Graph displays wing bud growth over the ten day experiment in reference to the control, low ethanol and high ethanol exposed chick embryos. Results conclude that there could be an effect on ethanol and wing bud length.

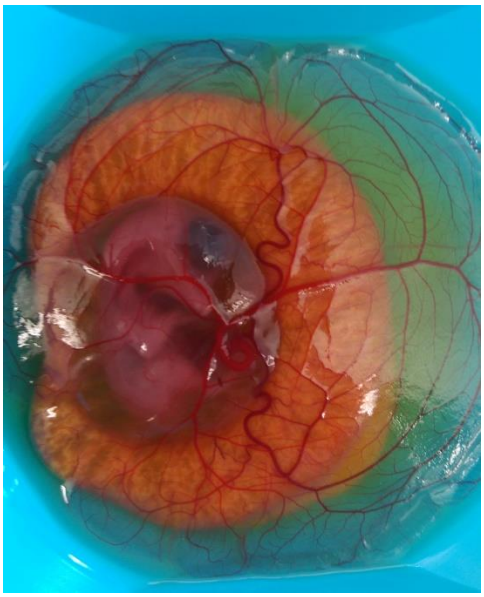

Figure 2: This image shows a high concentration 10 day chick embryo in the weigh boat. Acknowledgements to Carly Tavares for the image.

Results for wing bud length and ethanol exposure to chick embryos concluded that there may be an effect on growth. There can be no definite conclusions for low concentration of ethanol presented to chick embryos and wing bud length, because the data was averaged with only one embryo. The lack of embryo diversity and small sample size might have hindered the possible conclusions between ethanol and wing bud length. The data found for low ethanol concentration is still note-worthy, because the embryo's wing bud length might be affected by the ethanol. However, this conclusion cannot be made because of the small sample size. The graph shows the control embryo's wing bud length continued to grow over the 10 day observation period. The data also shows that the high concentration embryo's wing bud length grew laterally over the 10 day observation period. These results do not form a definite conclusion that ethanol hinders or enhances wing bud growth in chick embryo. However, there could still be a correlation between wing bud growth and the presence of ethanol.

Discussion

Ethanol exposure to chick embryos may affect wing bud length. The data collected showed no clear difference between chick embryo wing bud lengths affected by various ethanol concentrations. However, there still could be a possibility that ethanol plays a role on wing bud development in chick embryos.

My hypothesis that ethanol exposure to chick embryos reduces wing bud length, perhaps because of *Shh* signaling pathway might be hindered, was not supported. The results I found were not expected because of the literature on *Shh* signaling pathways. According to (Chiang *et al*, 2000), *Shh* is thought to be the key signaling protein for limb development. Chiang found that *Shh* is necessary for normal limb development for elbows and knees in chick and mice embryos. Knowing these findings, I thought the chick embryos treated with ethanol would have decreased wing bud growth because alcohol exposure reduces development. However, my findings did not

support my hypothesis or original thoughts. Ethanol may have an effect on wing bud length, but based on the results of the experiment, there can be no definite conclusion.

For future experiments, I think a larger sample size of chick embryos treated with alcohol and control embryos will show more conclusive results. Also, more embryos are needed when averaging length measurements due to embryos perishing, thus affecting the validity of the data set.

References

Ahlgren, S. (2002). Sonic hedgehog rescues cranial neural crest from cell death induced by ethanol exposure. Proceedings of the National Academy of Sciences, 10476-10481.

Armstrong, P.B., C.A. Erickson, R.D. Grey, and J.K.A. Metzler. (1994) Explant culture of chick embryo. in A laboratory text for developmental biology. 2nd ed. Kendall/Hunt Publishing Co. Dubuque Iowa. pp 83-86.

Chiang, C., Litingtung, Y., Harris, M., Simandl, B., Li, Y., Beachy, P., & Fallon, J. (2000). Manifestation of the Limb Prepattern: Limb Development in the Absence of Sonic Hedgehog Function. Developmental Biology, 236(2), 421-435.

Morris, R.L. (2015) Lab 6 – Chick in a boat model of fetal alcohol syndrome, 3/24/2015. Retrieved on [insert date you checked the webpage] from http://icuc.wheatoncollege.edu/bio254/2015/ChickInABoatProtocol_LabSheet_2015.htm

Zagory, J. (2004). Single early dose of ethanol causes acute morphological defects in the chick embryo: A study of Fetal Alcohol Syndrome. Retrieved April 28, 2015, from http://www.swarthmore.edu/NatSci/sgilber1/DB_lab/Student/FAS/FAS.html