Teratogenic effects of embryonic alcohol exposure on the limb buds of chicks (*Gallus gallus)*

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Introduction:

The study of abnormal development, also known as teratology, has become an increasingly interesting area of study as drugs and pollutants have become more abundant throughout the world. Fetal alcohol syndrome (FAS) has become a common disorder that is caused by the ingestion of alcohol during pregnancy; some of the common symptoms of this disorder include abnormal facial features, growth issues, and central nervous system problems (Li et al., 2007). A protein known as sonic hedgehog (Shh) is located in a signaling center known as the zone of polarizing activity (ZPA); Shh is known to be responsible for organizing the anterior-posterior axes of the limbs as well as for signaling for the expression of a protein known as FGF8 (Pownall & Isaacs, 2010). The FGF8 protein is crucial for proper proximal-to-distal, or outgrowth, of limb development (Pownall & Isaacs, 2010). It has been shown that when an organism is exposed to alcohol during its early development, the Shh signal transduction pathways are inhibited (Li et al., 2007). Therefore, with Shh signaling pathways inhibited, the FGF8 protein will not be signaled for expression, resulting in decreased outgrowth of limbs during development. In this study, we tested the effects of embryonic exposure to alcohol on chicks (*Gallus gallus)* to test the hypothesis that if a chick is exposed to alcohol, then it will develop shorter limb buds in comparison to unexposed chicks.

Materials and Methods:

Embryo Explanting

With some modifications, ten-day chick embryos (*Gallus gallus)* were explanted using the procedures given by Morris (2015) and Armstrong et al. (1994). One modification was that weigh-boat dishes were also used in place of Dixie cups fitted with plastic wrap; the dishes were placed inside petri dishes that were each labeled on the lid and side as "control", "0.02% alcohol", and "0.002% alcohol". Ethyl alcohol was administered to each corresponding 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-cell of 100 U mg/ml (Morris, 2015).

Data Collection

Continuing to follow the procedure provided by Morris (2015), measurements and observations were made over 8 days, allowing me to document chick development between 3 and 10 days old in order to observe the effects of alcohol on early chick development. The

measurements that were recorded on a given data sheet included pulse (measured in heart beats/min), the diameter of the Area Vasculosa (along the longest axis), the body length (along the longest axis), the wing and leg bud lengths (from base to tip), and the allantois width (along the longest cross axis). Measurements were made either in millimeters with a ruler or using a microscope that contained a ruler within its eyepiece; magnification was recorded on the data sheet in order to convert the microscope unit measurements into millimeters after data was collected. Data that was collected from a total of 15 people was compiled and used in the final analysis of the effect of alcohol on chick development.

Results:

The wing and leg bud length measurements that were collected were averaged on each day from the data that was available. Due to unsuccessful explanting of chick embryos and death of embryos early on in the experiment, there was very minimal data collected from chicks exposed to 0.002% alcohol (low ethanol dose) and they were therefore excluded due to lack of data. Typically, measurements of wing and leg bud lengths could not be made until Day 5 because the chicks were too premature prior to Day 5 to have developed visible buds; therefore, the data shown in Figures 1 and 2 describe average lengths measured from Day 5 to Day 10 of chick development. As shown in Figure 1, chicks that were exposed to the high concentration of alcohol contained larger wing buds day-to-day in comparison to the control chick embryos. On the final day of measurements, Day 10, the chicks exposed to the high concentration of alcohol on average had a wing bud length of 6.343 millimeters, while the control chicks on average had wing bud lengths of 6.225 millimeters. As shown in Figure 2, leg bud length was also shown to be larger in the alcohol-exposed chicks from day-to-day in comparison to the control chicks. On Day 10, the alcohol-exposed chicks on average had leg buds that were 11.089 millimeters in length while the control chicks on average had leg buds that were 9.36 millimeters in length.

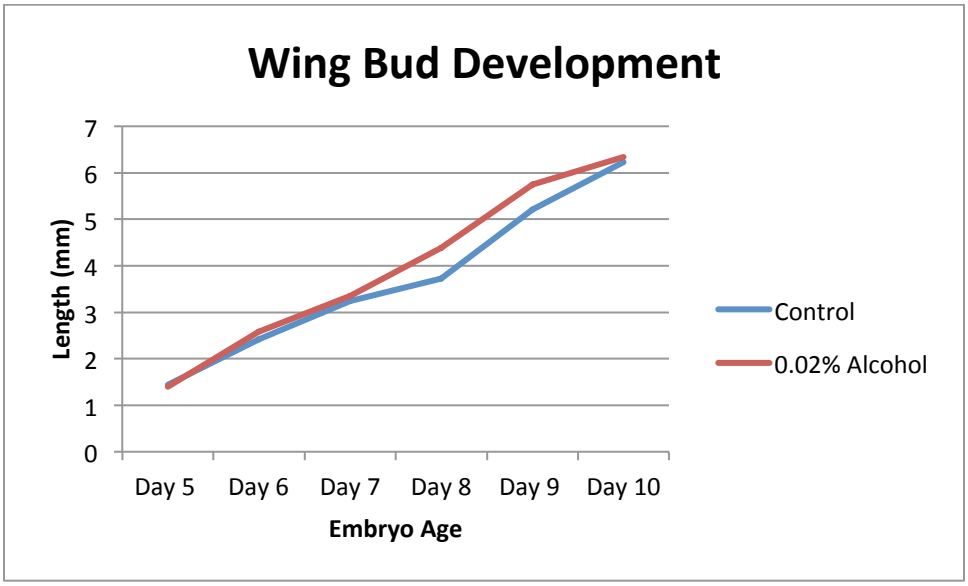

Figure 1: The average wing bud lengths of chick embryos (*Gallus gallus*) between Day 5 and Day 10 of development. The blue line represents chick embryos that served as the control and were not exposed to alcohol. The red line represents chick embryos that were exposed to 0.02% ethanol (high ethanol dose).

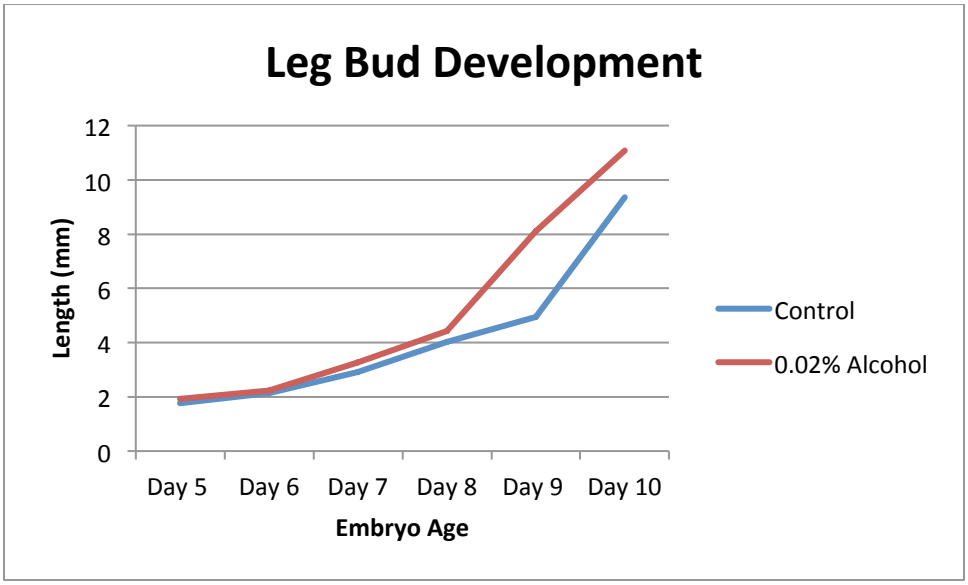

Figure 2: The average leg bud lengths of chick embryos (*Gallus gallus*) between Day 5 and Day 10 of development. The blue line represents chick embryos that served as the control and were not exposed to alcohol. The red line represents chick embryos that were exposed to 0.02% ethanol (high ethanol dose).

Discussion:

This data concludes that exposure to alcohol does not decrease limb bud outgrowth and therefore, does not support the hypothesis that chicks exposed to alcohol will develop shorter limb buds in comparison to chicks that were not exposed to alcohol. Other research has

concluded that alcohol exposure during early development induces a dose-dependent inhibition of hedgehog signaling pathways, which in turn will prevent the expression of FGF8 proteins (Pownall & Isaacs, 2010). The FGF8 protein has been shown to be crucial in limb development and outgrowth. By limiting the FGF8 protein in chick embryos, researchers have concluded that limb bud development will be abnormal, resulting in shorter limb buds (Lewandoski, Sun, & Martin, 2000). Instead, this set of data suggest that exposure to alcohol may actually promote limb bud outgrowth, especially when referring specifically to leg bud development. These results were not expected and do not agree with the conclusions that have been reached by other researchers. I believe that future experiments should include a larger number of chick embryos in order to collect a more sufficient amount of data to reach a well-supported conclusion; due to the amount of explanting failures and early embryo deaths, there were a limited number of embryos from which data was collected from over all 10 days of the experiment. I would also propose using a larger range of alcohol exposure in future experiments; it is possible that 0.02% alcohol (high ethanol dose) was not sufficient enough to inhibit the hedgehog signaling pathways to the point where FGF8 expression was affected.

References:

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