Fetal alcohol syndrome on notochord induction in chick embryos

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Introduction:

Fetal alcohol syndrome describes a diversity of phenotypes that result from early ethanol exposure in embryonic development. The array of these developmental abnormalities ranges from physical deformities of the face (5), notochord (3), and limb formation (4). Notochord defects, in particular, are a critical developmental error for vertebral organisms. Not only is the notochord the distinguishing feature of the phylum Chordata, but it also serves an essential role in developmental induction since it contains singling molecules necessary for the formation of muscle and skeletal structures (6). Among the many signaling molecules present in the notochord is sonic hedgehog (shh), a member of the hedgehog family of signaling proteins, which is responsible for inducing proper somite formation and vertebral development that will later become the spine (3). Studies have demonstrated that the transcription of shh and is activator proteins is significantly reduced in the presence of ethanol (5). The disruption in the sonic hedgehog signaling pathway perturbs proper notochord induction, and the associated phenotype with this molecular pathway is deformed somites and reduced stature (3).

This experiment investigated the relationship between ethanol exposure in early chick development and physical deformities in the notochord. Particularly, this experiment measured body length as a benchmark for notochord development. The hypothesis for the study was that ethanol exposure to chick embryos would result in a lower average body length than the control.

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 (1). 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) (1). Penicillin/streptomycin was also added to each embryo to achieve a final concentration on-the-cells of 100 U mg/ml (1). The ethanol was diluted in Tyrode's solution, which was also the only chemical introduction to the control.

Following the addition of ethanol to the experimental groups, the embryos were observed over the course of ten days under a dissection scope. Measurements were taken of the body length, pulse, area Vasculosa diameter, wing bud length, leg bud length, and allantois width, although only the data pertaining to body length would be analyzed in

this report. Body length was measured from the tip of midbrain to the tail of the embryo. In addition to this quantitative data, qualitative observations were also made about the embryo's development over time. After a period of twelve days post fertilization, the embryos were terminated in accordance with IACUC protocol.

Results:

Fig. 1: Image of an Eight-Day-Old Chick Embryo Exposed to 0.02 % EtOH

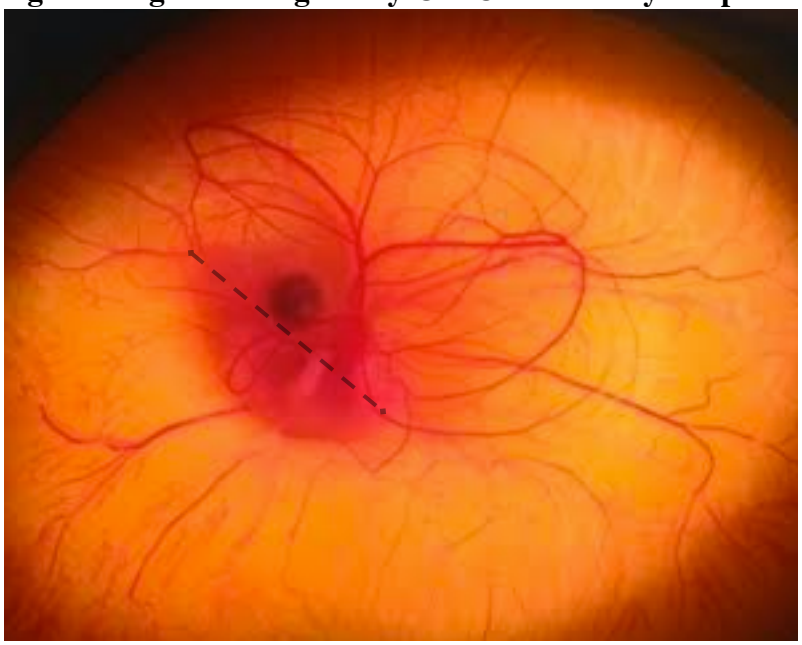

Fig. 1: Above is a photograph of an eight-day-old chick embryo exposed to 0.02% ethanol, taken without magnification. The most evident structures at this developmental stage include the eye, wing and leg bud, midbrain, allantois, and area Vasculosa. The dashed line overlaying the chick embryo depicts the dimension of measurement taken of body length that was used to collect the data; this line extends from the tip of the midbrain to the tail of the embryo.

Fig. 2: Effect of Ethanol Dose on Average Body Length Over Time

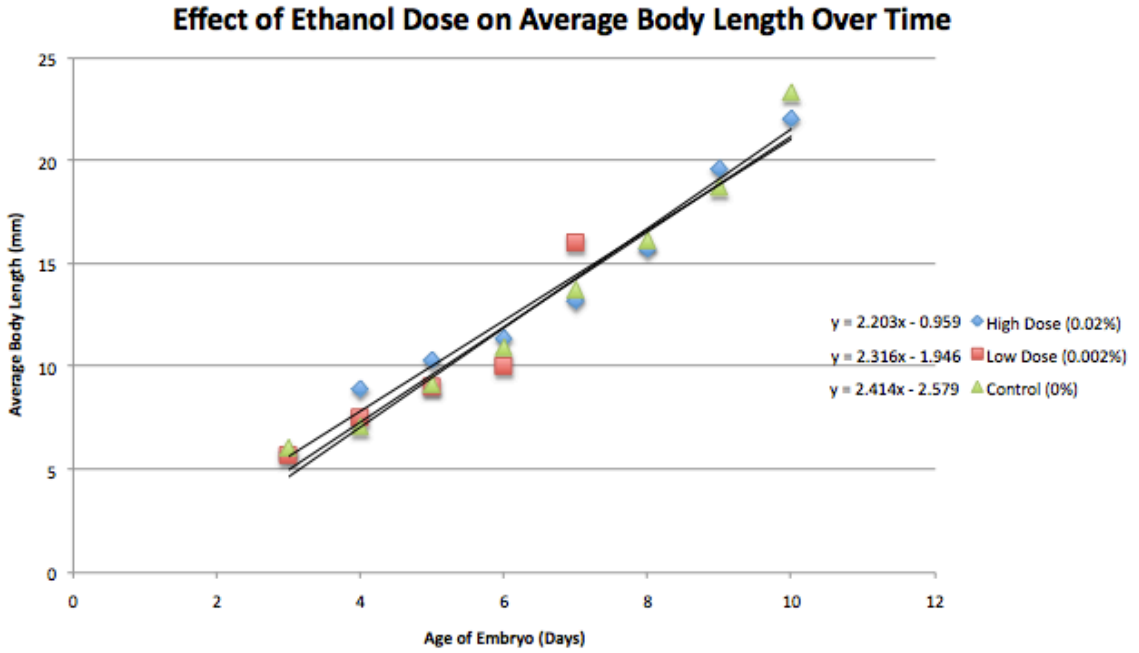

Fig. 2: This chart plots the relationship between the age of the embryo in days and the average body length, measured in millimeters. Three lines are plotted, each representing a different condition of ethanol concentration. The control group, depicted by the green triangles, contains 0% EtOH, the low dose experimental group, depicted by the red squares, contains 0.002% EtOH, and the high dose experimental group, depicted by the blue diamonds, contains 0.02% EtOH. The body-length measurements were taken starting on day three of development, and for the control and high dose experimental group, these measurements terminated on day ten. Because no embryo in the low dose experimental group survived past day seven, the measurements for this condition end here. Finally, a line of best-fit has been included to describe each of the relationships between time and body length for the three groups, and its linear equation can be found to the right of the chart corresponding to the group it represents. It should also be noted that the sample size for the control group was 13, for the low dose experimental group, the sample size was 3, and for the high dose experimental group, the sample size was 12.

Figure 2 displays a positive linear relationship between the two ethanol experimental groups and the control group, where average body length is plotted over time in days. The slope of the best-fit lines for each of the groups is nearly identical, all within 0.1 of one another. This strong similarity indicates that the increase in body length over time as displayed on the y-axis does not significantly vary among between the experimental and control conditions. The slight deviations observed in individual data points along the line of best-fit are likely attributed to random variation or experimental error. It should also be noted that the data collected for the low dose experimental group is incomplete, as no embryos survived passed day 7. Though this limits the predictive value of the line of best fit, it seems to comply with an almost indistinguishable trend from the other two groups. Overall, these data indicate that ethanol exposure even in its

highest concentration for this experiment does not significantly affect embryo body length.

Discussion:

The results gathered from this lab do not support the hypothesis that the embryo body length will be reduced under exposure to 0.002% and 0.02% ethanol. The experimental groups did not vary significantly from the control, and no relationship between ethanol dose and body length has been demonstrated in even the highest of the ethanol doses. This result contradicts the current literature on FAS and its affect on body length; a study by Evyn Loucks and Sara Ahlgren investigating the teratogenic effects of alcohol on zebrafish found that zebrafish length was significantly reduced as ethanol exposure increased early in development (8). In addition to using zebrafish as a model organism for FAS, the Loucks and Ahlgren study exposed the embryos to concentrations of ethanol ranging from 1.5% to 2.5%, which is a range much higher than concentrations of ethanol in used in this experiment. The conclusion of this lab is that 0.02% and 0.002% ethanol was insufficient to induce FAS as measured by body length could be due to the very low concentrations of ethanol administered to the chick embryos. In another study investigating the effect of ethanol on developing skeletal muscle of chick embryos, JD Chaudhuri found that 5% to 15% ethanol concentrations were sufficient to induce musculoskeletal defects in the chick embryos (7). Future experiments to test the effects of FAS on notochord induction in chick embryos should be preformed with increased concentrations of ethanol in the experimental groups, perhaps between the range of 2% to 15%. These higher doses will maximize the chance of observing an effect in the experimental groups.

In addition to increasing the ethanol concentration, future experiments could also investigate the effects of periodic ethanol exposure on development. Varying ethanol exposure on a periodic schedule would more closely model the actual conditions of FAS in human populations, where maternal blood alcohol concentration is not a constant value. As such, an experiment could more accurately predict the alterations in development and molecular pathways related to maternal drinking in early development.

References:

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