Activity #1 – Observing Morphological Changes in Embryonic Development

Observations: The morphology of the chick embryo is a rapid process directed by several factors, including genetic, temporal-spatial molecular regulation, and the mechanical environment. When conducting an experiment, choosing what to measure is an important, and sometimes difficult, decision. To help with deciding, potential data is classified into different types. One such classification is quantitative vs. qualitative.

Q. #1 - “Science Speak”: What’s the difference between quantitative and qualitative observations?

Quantitative –

Qualitative -

Q. #2 – What are three possible qualitative and quantitative observations we can make using the ex-ovo culture system?

Data Collection: For our study of the effects of retinoic acid (RA) on the developing embryo, we are going to use two morphological parameters to compare between treatments. The first is Tip-to-Tail length, defined as the longest line drawn between any two points in the embryo. As previous research has suggested that excessive vitamin A will stunt development, Tip-to-Tail length will serve a measure of overall embryo growth. Secondly, we will measure eye diameter as retinoic acid is also implicated in eye defect formation. As you can imagine, there are several other possibilities, but these should yield informative results that fit within our resources of both time and equipment. Research resources are an important consideration when designing an experiment.

Use ImageJ to measure the Tip-to-Tail length and eye diameter of the RA case study data in the file titled “RA_Exp_Data.tif”. Process the data as instructed, and answer the data analysis questions. Before taking a measurement, be sure to calibrate the image to 6.25µm/pixel, under Image>Properties.
Record the tip-to-tail length for each experimental condition in table below. Be sure to calibrate image before taking measurements. **Note:** record data in millimeters (mm), therefore you will need to convert micrometers (µm) \( \rightarrow \) mm

<table>
<thead>
<tr>
<th>Tip-to-Tail Length (mm)</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td></td>
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<tr>
<td>Sham</td>
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<tr>
<td>Retinoic Acid (1mg/ml)</td>
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<tr>
<td>Retinoic Acid (2mg/ml)</td>
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</table>

Plot tip-to-tail length vs Day for all experimental conditions.
Record the eye diameter for each experimental condition in table below. Be sure to calibrate image before taking measurements. Note: record eye diameter data in micrometers (µm)

<table>
<thead>
<tr>
<th>Eye Diameter (µm)</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
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Plot eye diameter vs Day for all experimental conditions.
**Data Analysis:** After collecting the data, researchers must interpret it to gain insight into the phenomenon under study. Work through the following questions as you analyze the data.

**Q#1)** Compare the Tip-to-Tail length and eye diameter between the control and RA treatments. Did addition of retinoic acid stimulate or stunt growth?

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**Q#2)** Approximate the growth rate of each treatment by determining the slope of the Tip-to-Tail curves. Which condition had the fastest growth rate? Which was the slowest?

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**Q#3)** The 2mg/ml RA treatment only had data for Day 4-5, while the 1mg/ml RA treatment has data from Day 4-7. What does this suggest about the relative toxicity of the two dosages?

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**Q#4)** On Day 7, is the eye diameter of the RA 1mg/ml treatment greater or less than the control? Do you see any qualitative differences between the eyes of these two treatments?

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Q#5) Are the sham results different from the control? What do the sham results indicate about our method of treatment?

Q#6) From our data we see that RA treatment effected the *global* development of the embryo, yet the treatment was applied *locally*. Why do you think this is?

Q#7) In the last image of both RA treatment the vasculature (blood vessel network) appears to be receding. Why do you think this is happening, and what effect would this have on the developing embryo?

Q#8) Our initial motivation for studying retinoic acid excess in the embryo was to assist vitamin A deficient mothers and infants. How do our results inform corrective therapies for this condition?