

Effect of Gut Microbiota on the Enteric Nervous System of the Sea Cucumber

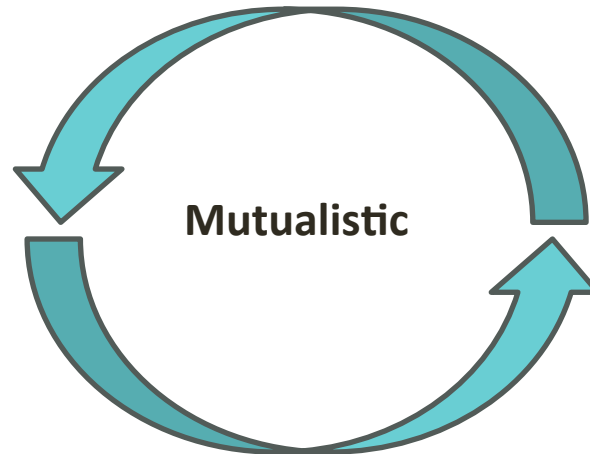
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Microbiota

- Symbiosis (mutualistic)
- All organisms have mutualistic relations.



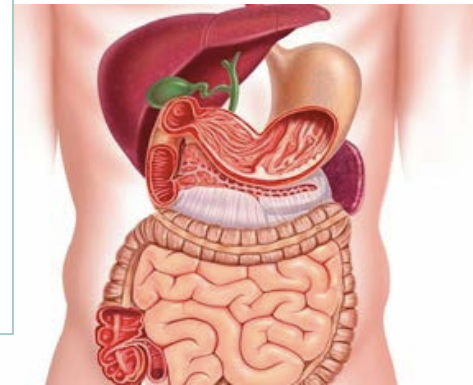
Gut Microbiota

- Crucial in essential physiological processes involved in homeostasis for different organisms.
- Also related to immune system and health in general.
- Responds to changes or alterations.

Gut Microbiota: the unknown organ that maintains our health

***Drosophila* Microbiome Modulates Host Developmental and Metabolic Homeostasis via Insulin Signaling**

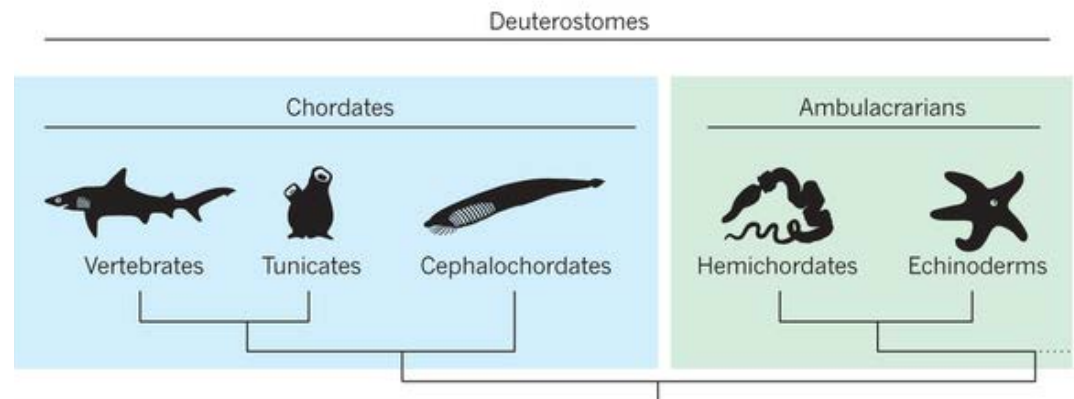
Seung Chul Shin *et al.*

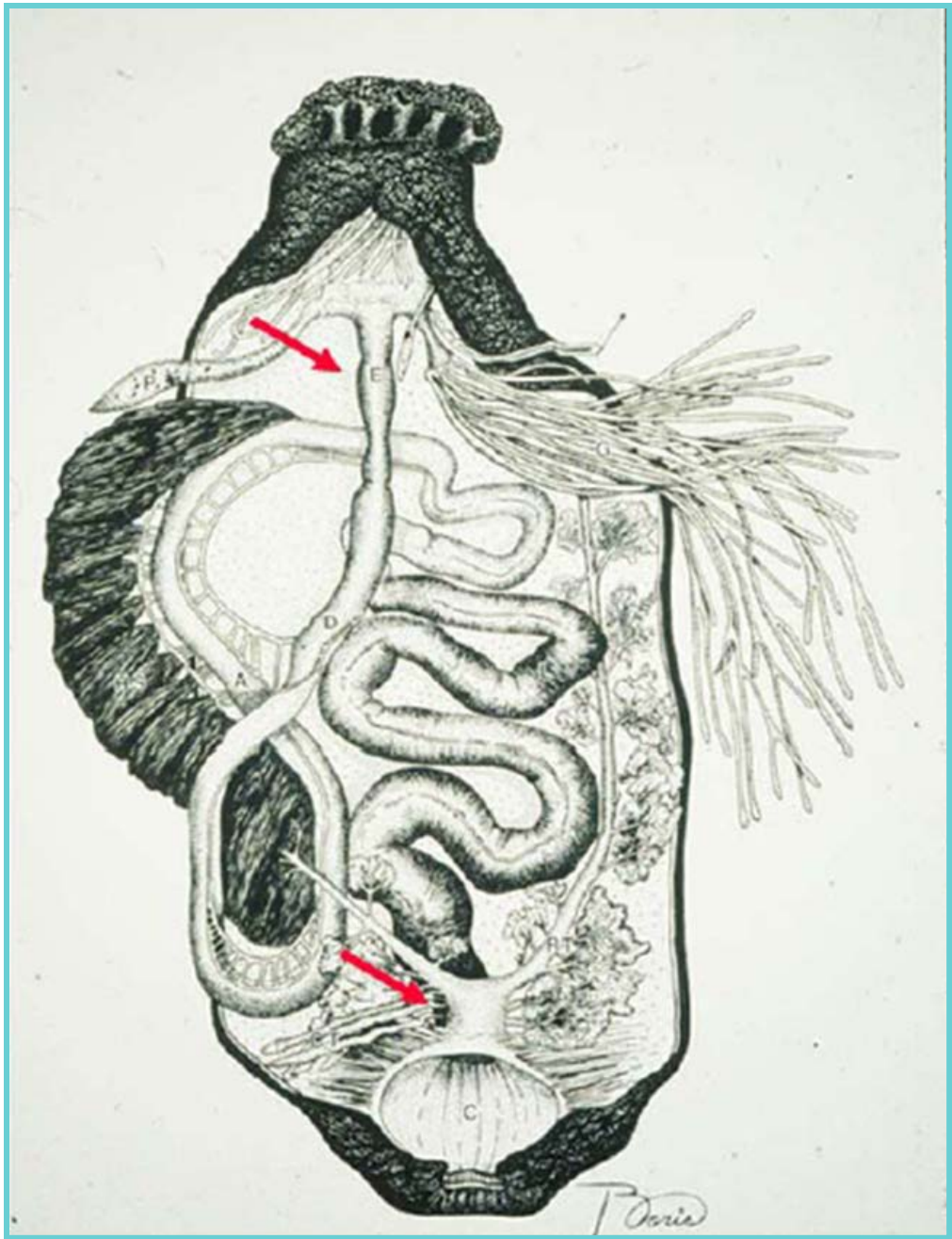


Gut Microbiota Are Related to Parkinson's Disease and Clinical Phenotype

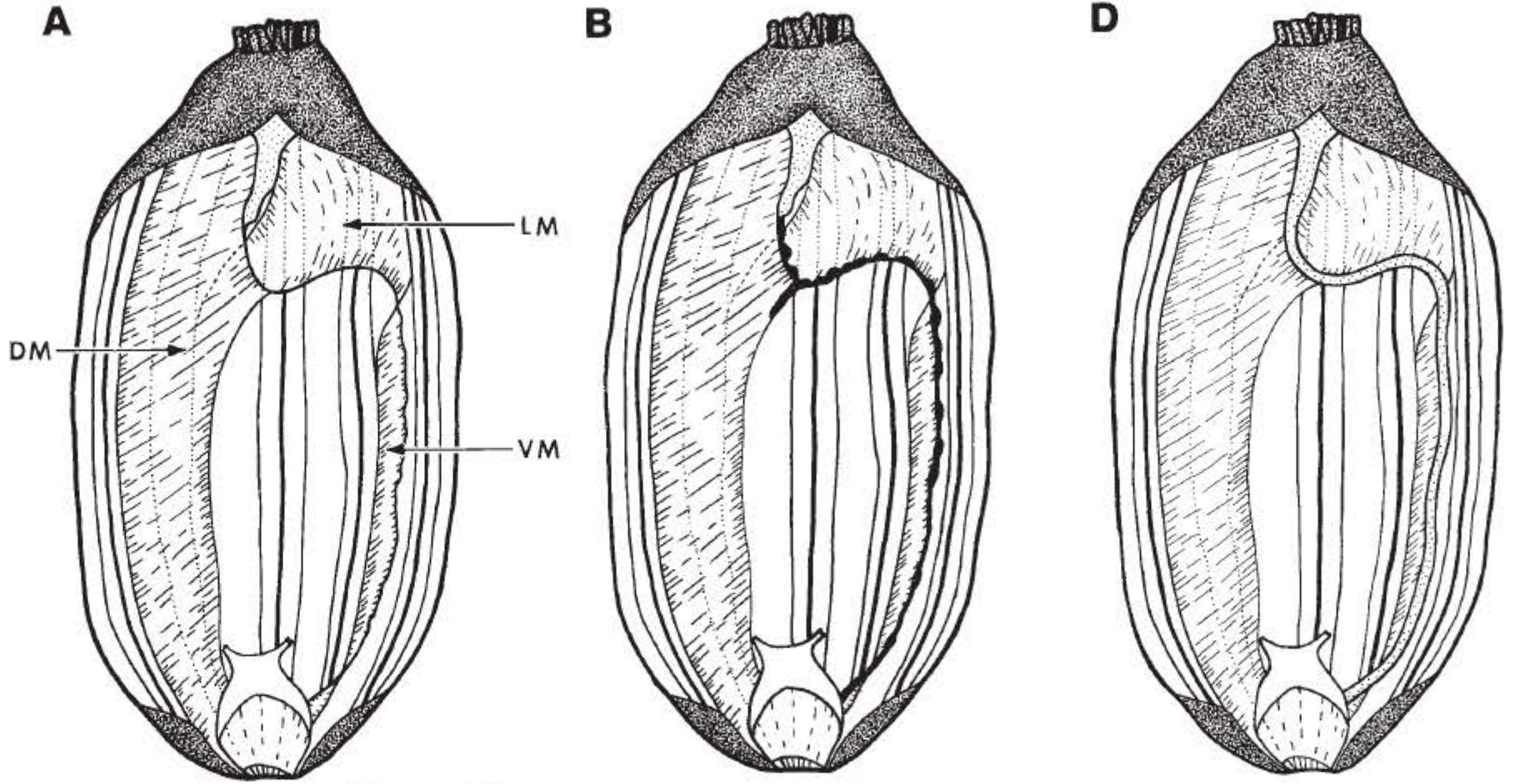
Scheperjans, Filip, et al.

Holothuria glaberrima

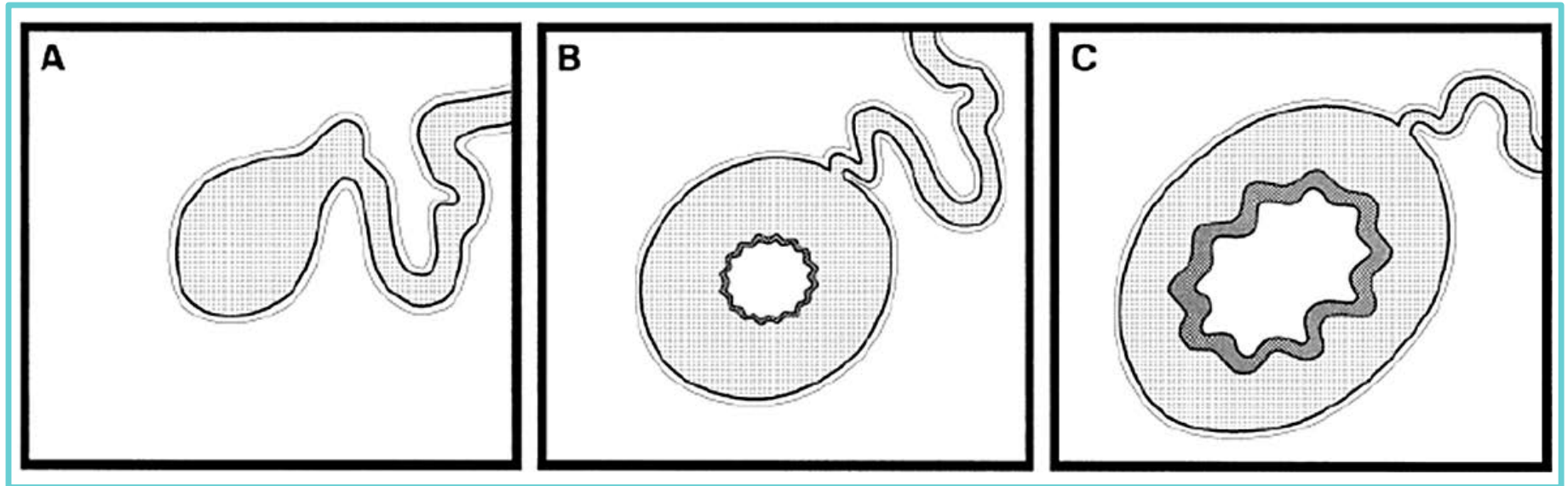




Holothuria glaberrima



Intestinal Regeneration

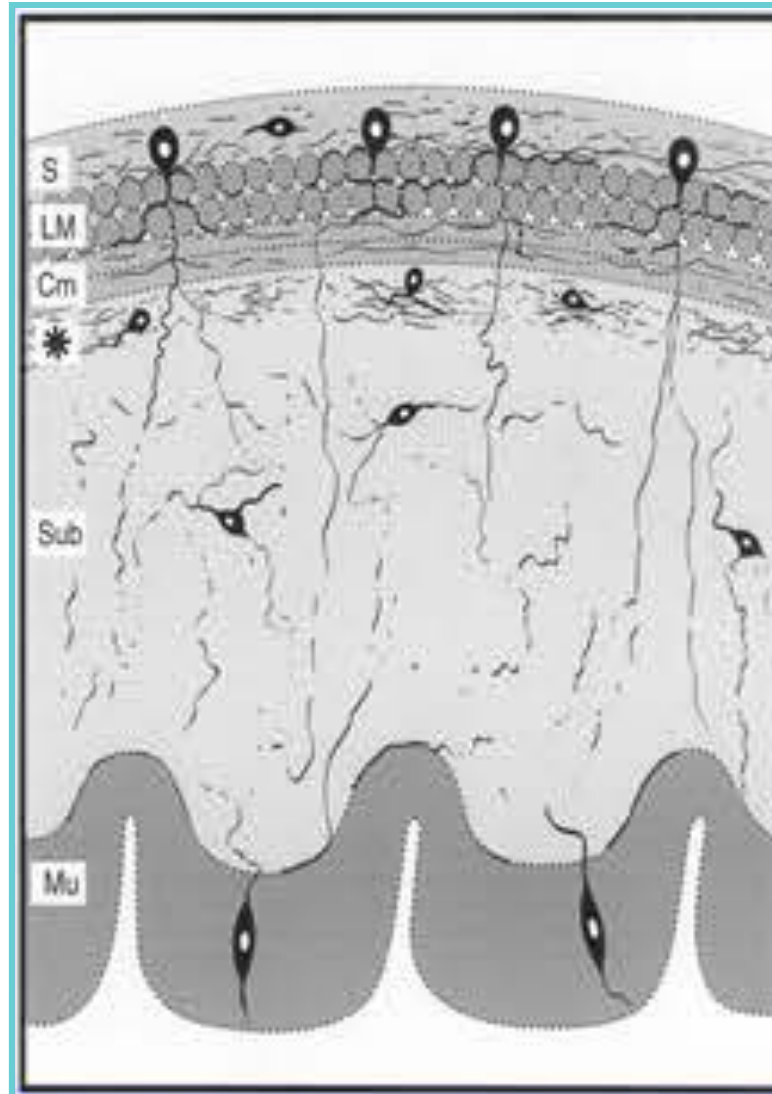


First week

Second week

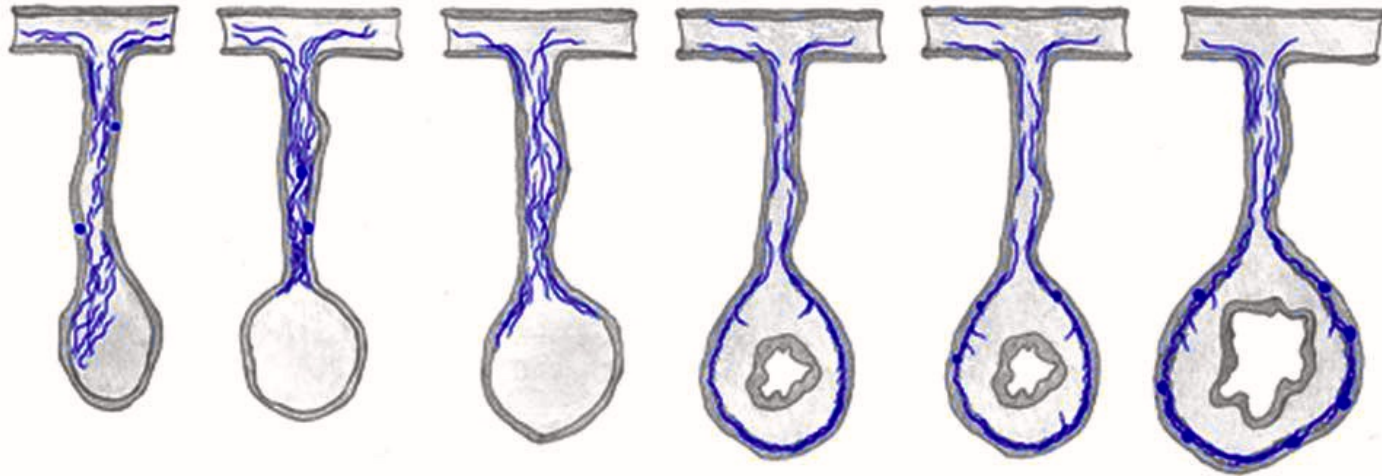
Third week

Nervous System Regeneration



Nervous System Regeneration

A



B



I
5-7 dpe

II
8-10 dpe

III
~14 dpe

IV
~21dpe

V
~28 dpe

VI
~35 dpe

Objective

Determine the effect gut microbiota on the regeneration of the intestinal tract of the sea cucumber *Holothuria glaberrima*.

- Antibiotic usage
- Measuring blastema size and regenerated intestine length
- Immunohistochemical analysis: RN1

Methodology



Methodology

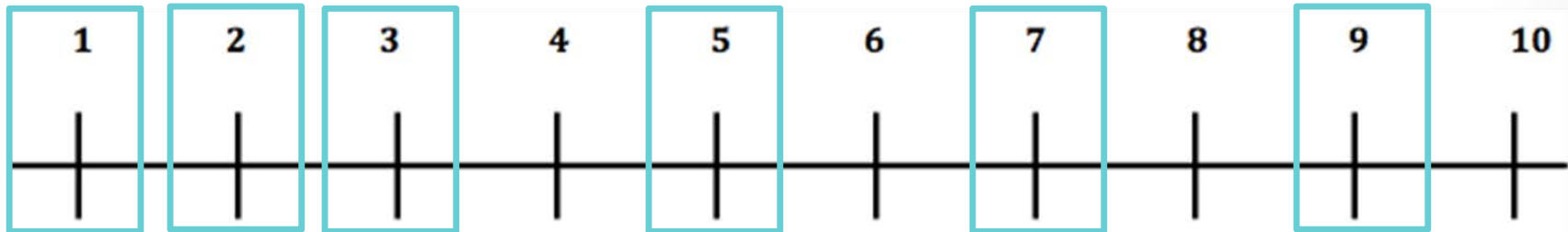
Antibiotic Treatment

Control Group (Artificial Seawater)	Penicillin/ Streptomycin (100 μ g/mL) and Kanamycin (100 μ g/mL)	Penicillin/ Streptomycin (100 μ g/mL) and Erythromycin (20 μ g/mL)	Penicillin/ Streptomycin (100 μ g/mL) and Neomycin (100 μ g/mL)	Penicillin/ Streptomycin (100 μ g/mL)
1L Water	1L Water 10mL Pen/Strep 1mL Kanamycin	1L Water 10mL Pen/Strep 1mL Erythromycin	1L Water 10mL Pen/Strep 10mL Neomycin	1L Water 10mL Pen/ Strep

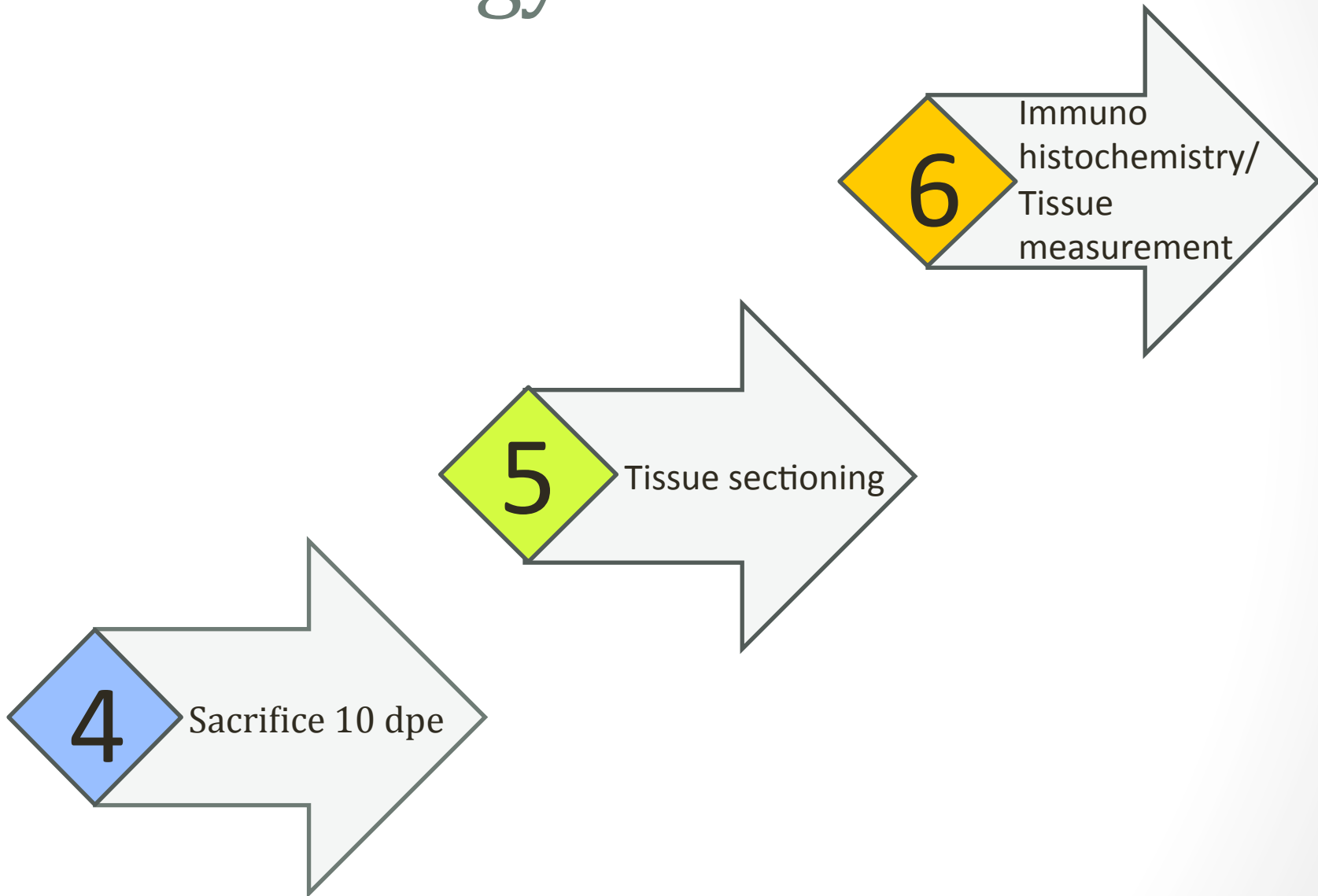
- 4 animals per group Total number of animals: **20**

Methodology

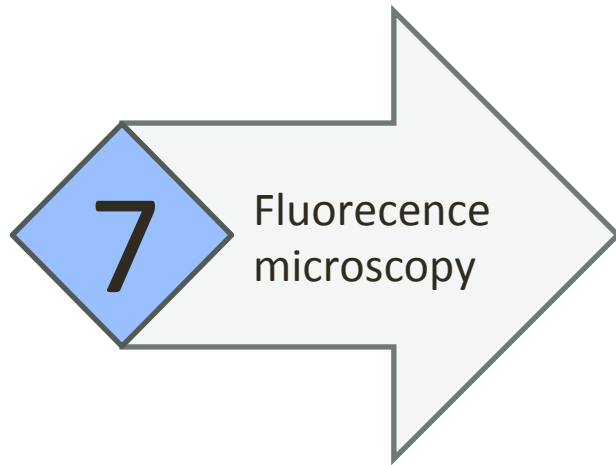
Antibiotic treatment: 10 days post evisceration



Methodology



Methodology



Measurement of Intestine Length

Anterior
Lumen Small
(Intestine)

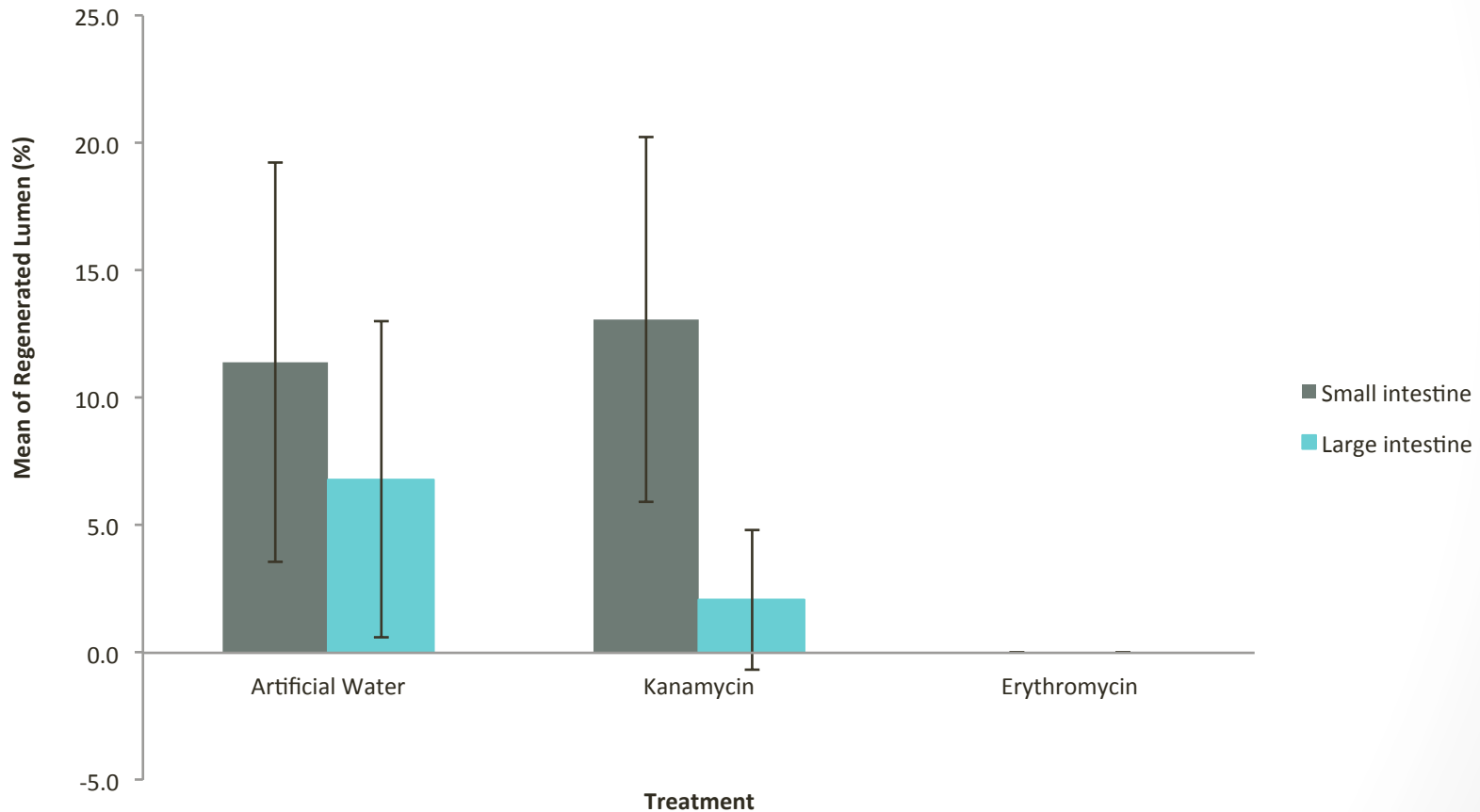


Posterior
Lumen Large
(Intestine)

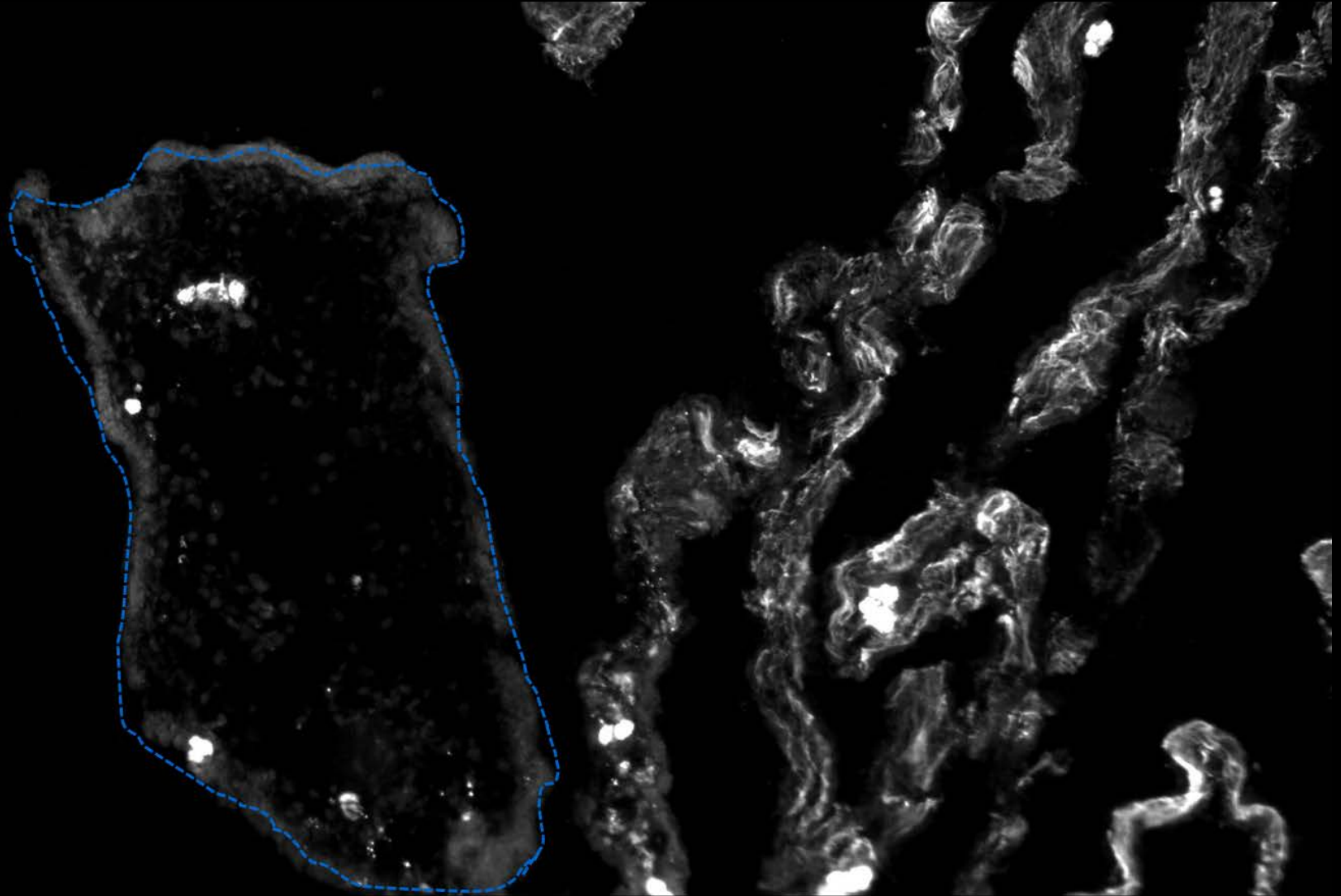
Whole Intestine

Results: Measurement of Intestine Length

Effect of Antibiotics on Lumen Formation

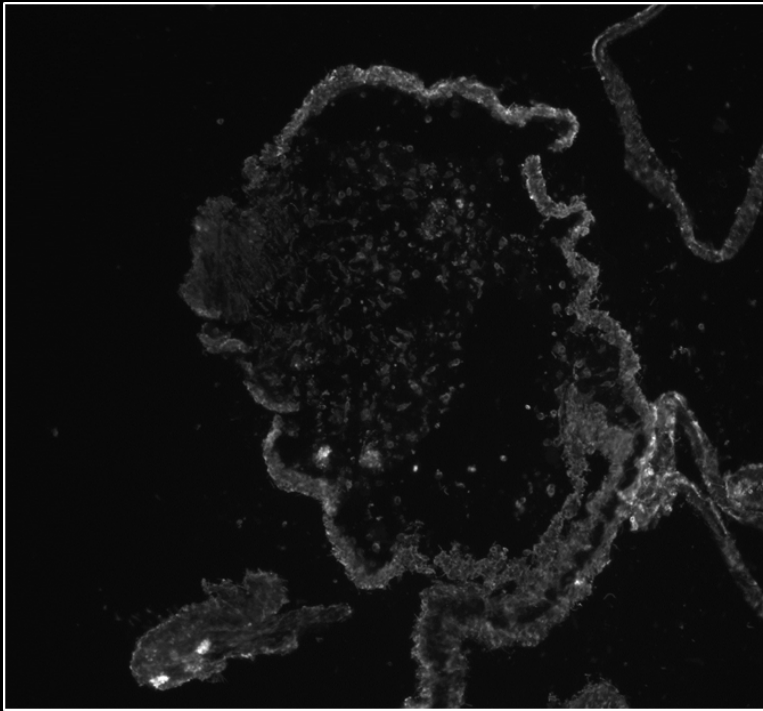


Measurement of Blastema Size

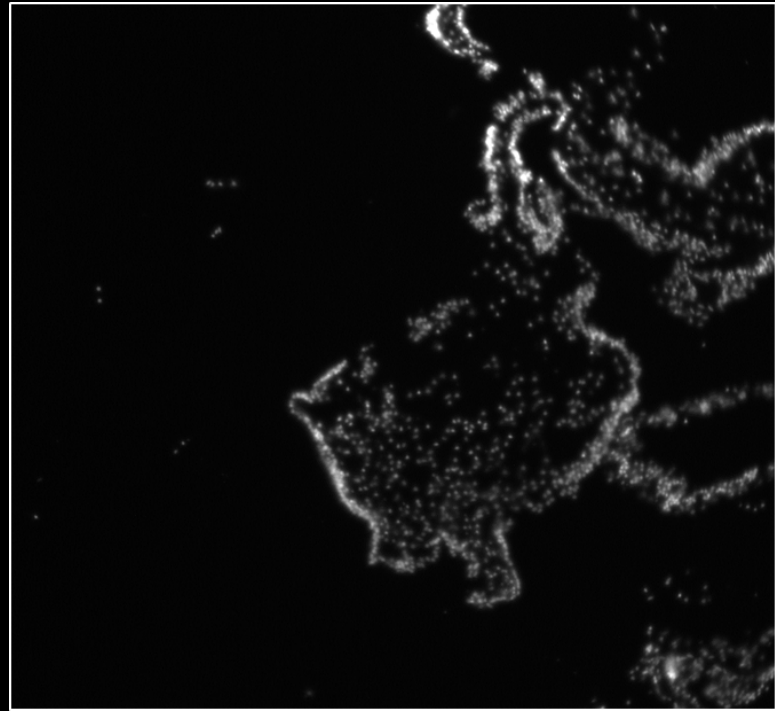


Control vs. Kanamycin

Control

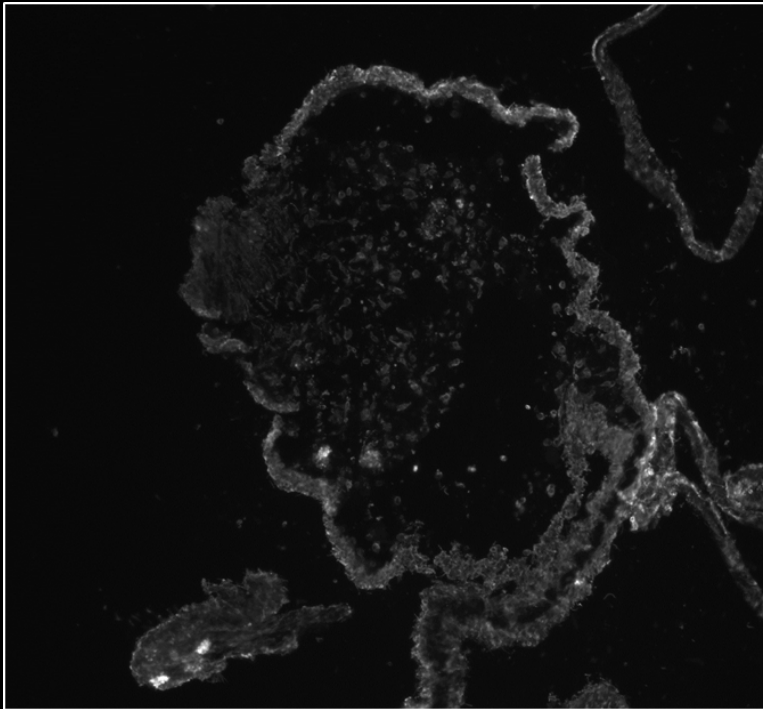


Kanamycin

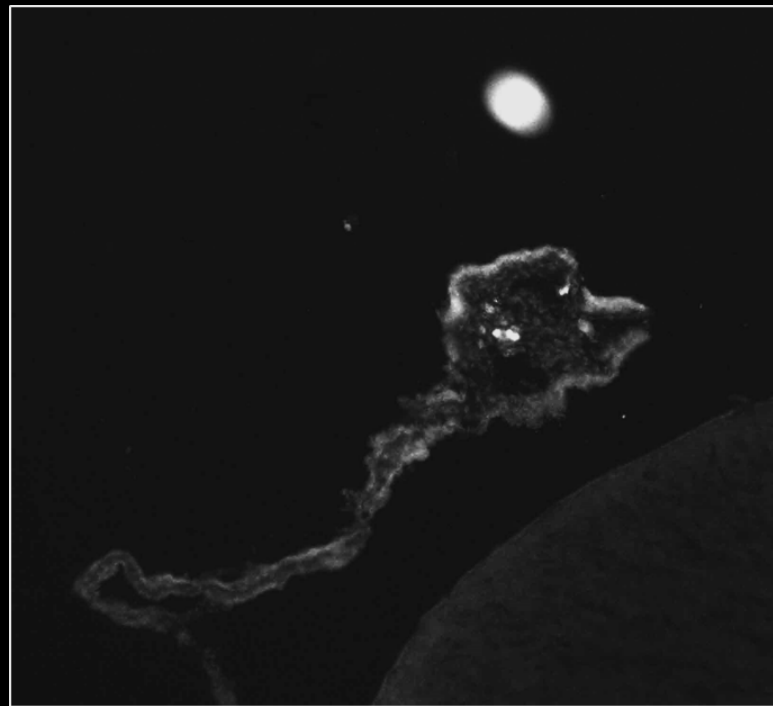


Control vs. Erythromycin

Control

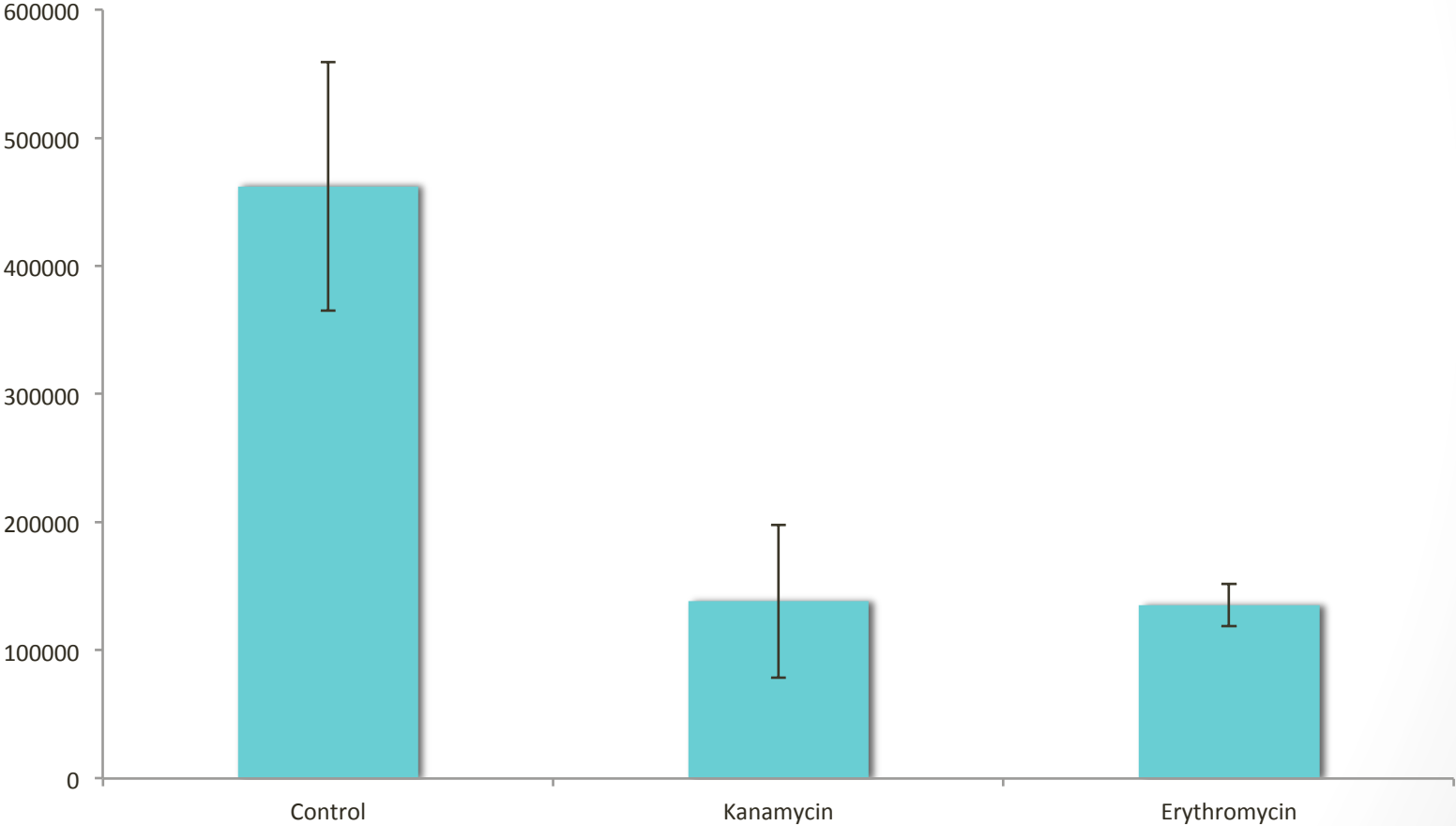


Erythromycin



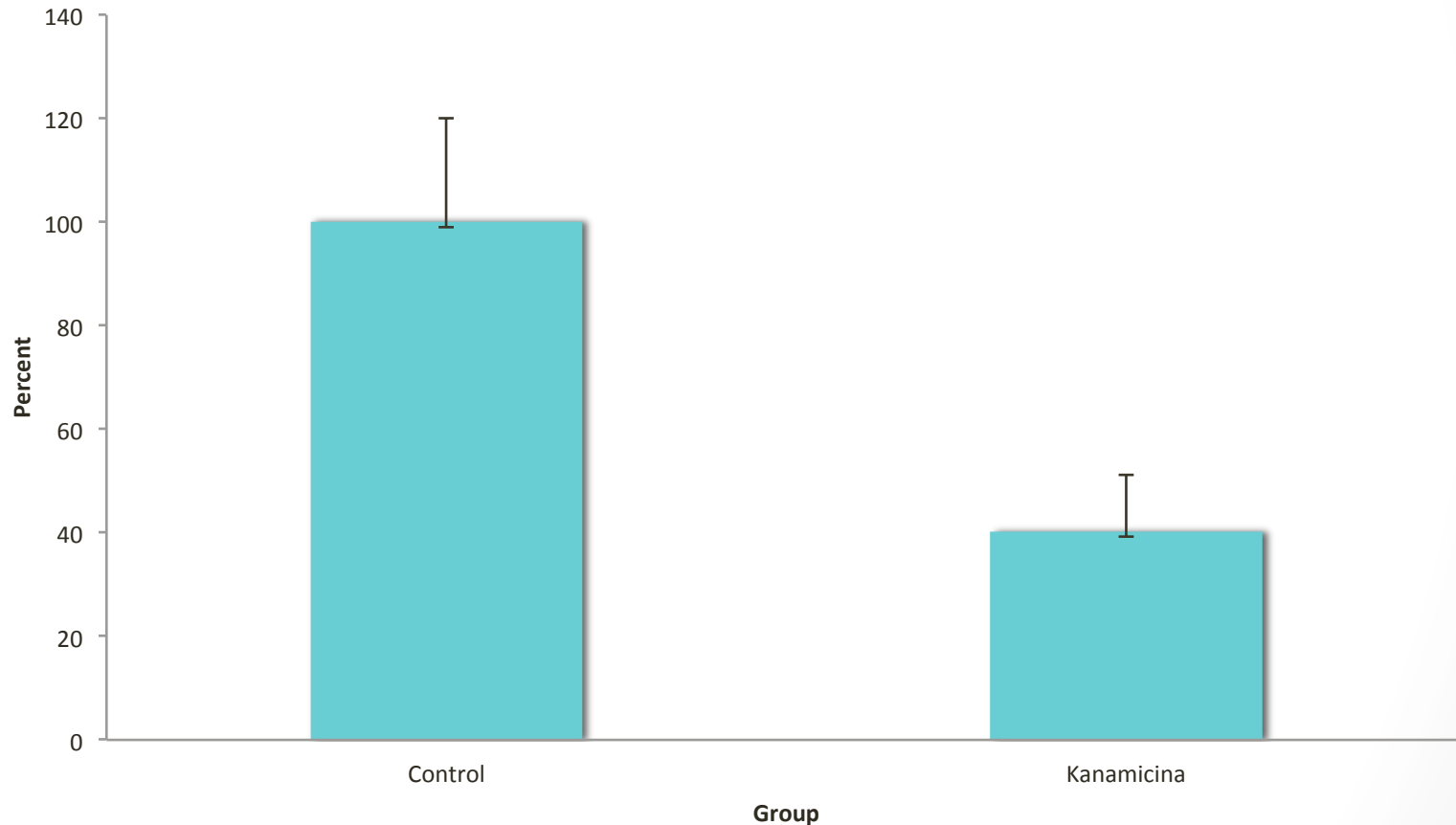
Results: Measurement of Blastema Size

Effects of Antibiotics on the Area of Blastema



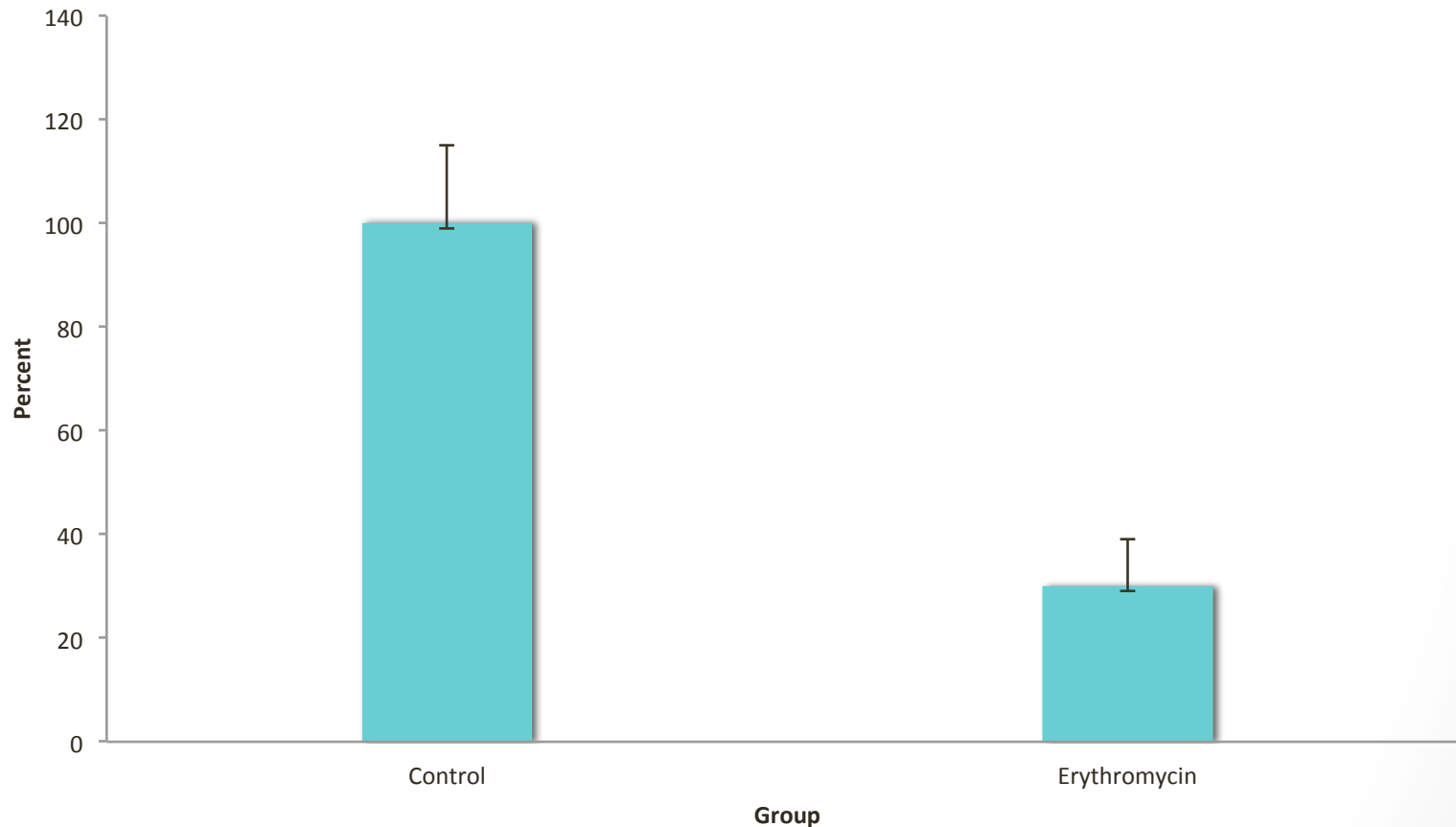
Comparison with Previous Experiments

Effect of the Antibiotics in Rudiment Size (%)



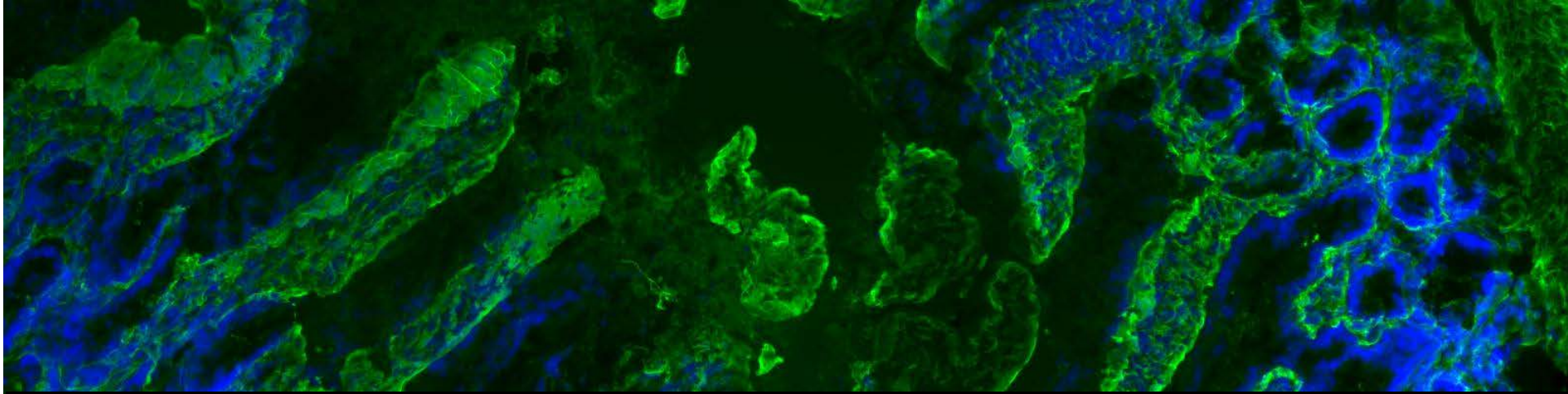
Comparison with Previous Experiments

Effect of the Antibiotics in Rudiment Size (%)

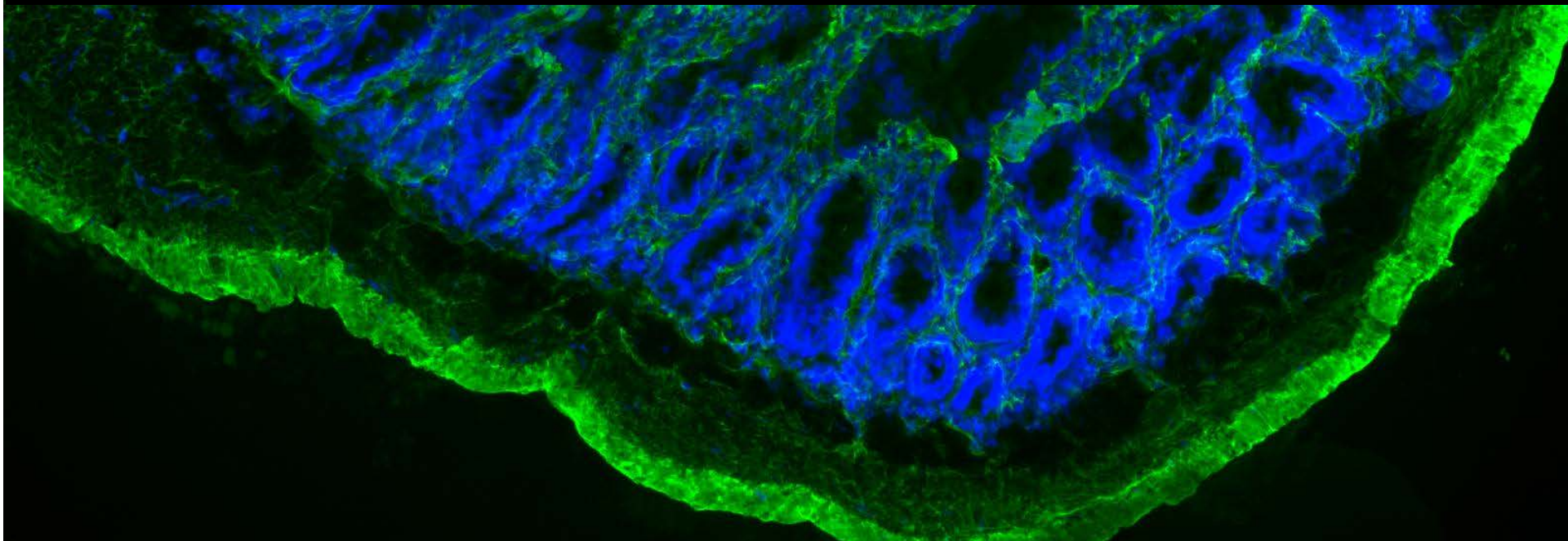


Observations

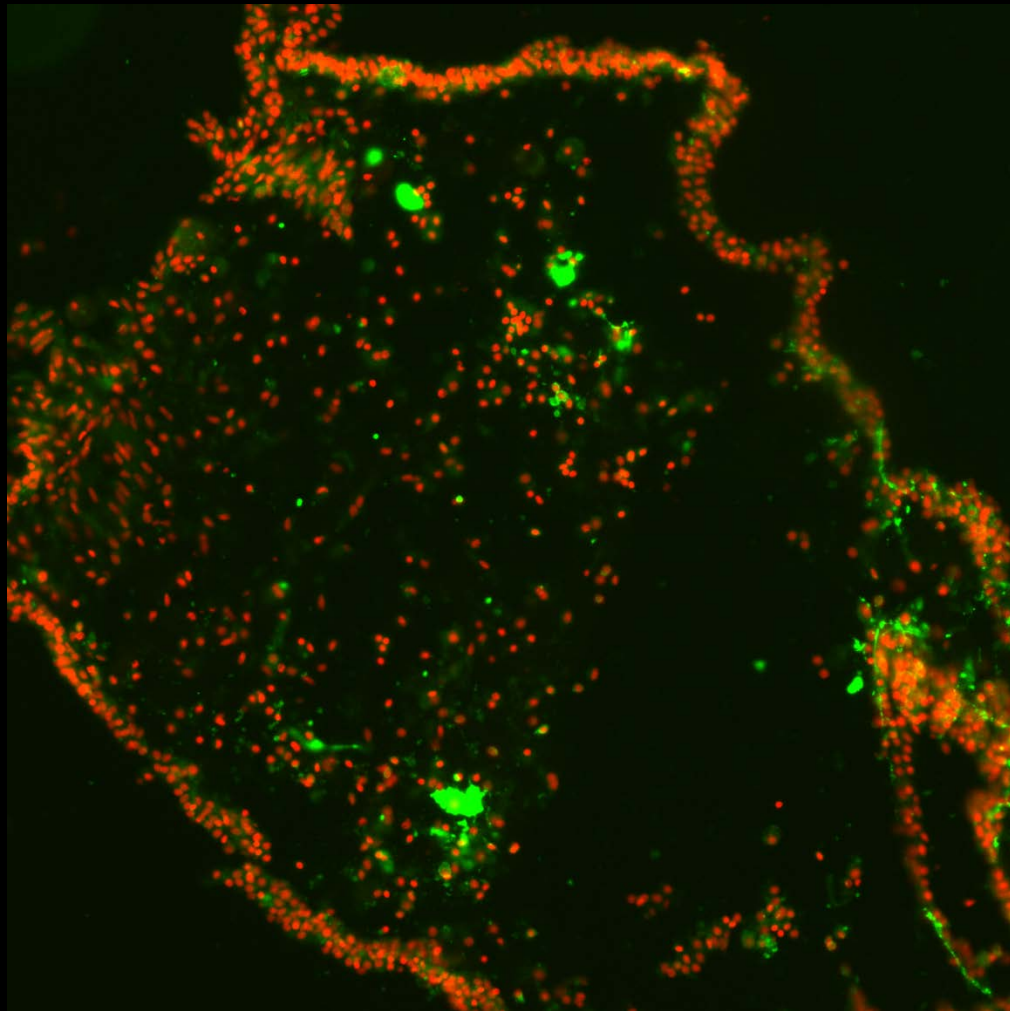
- As observed Kanamycin has no effect on lumen formation; however, there was an apparent effect on animals treated with Erythromycin.
- As seen in previous results, antibiotics have a negative effect on rudiment size.
 - Animals treated with Kanamycin showed a significant reduction in the size of the blastema.
- Recently, we have observed a smaller rudiment in animals treated with Kanamycin or Erythromycin .



Immunohistochemical analysis

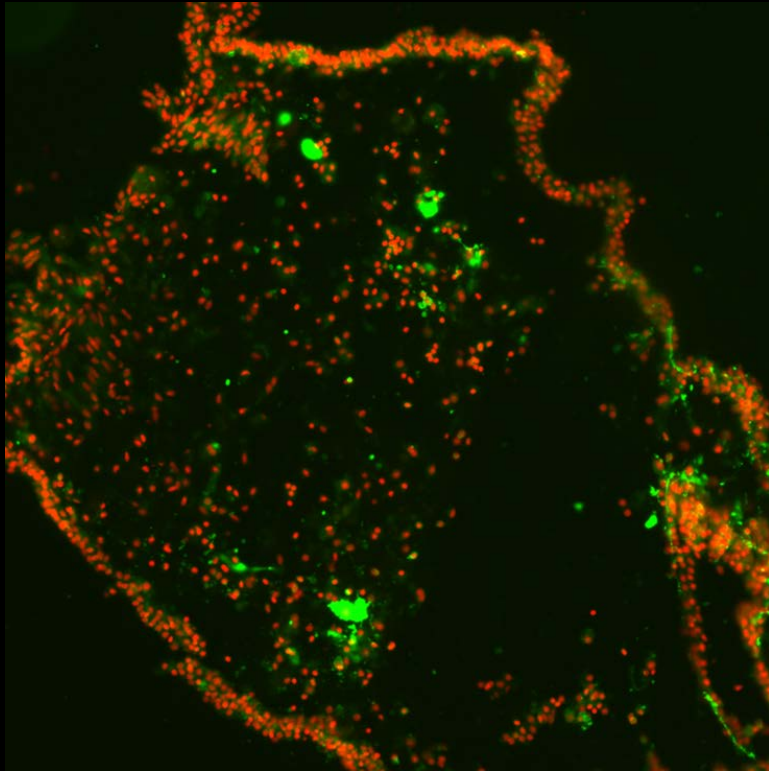


Control

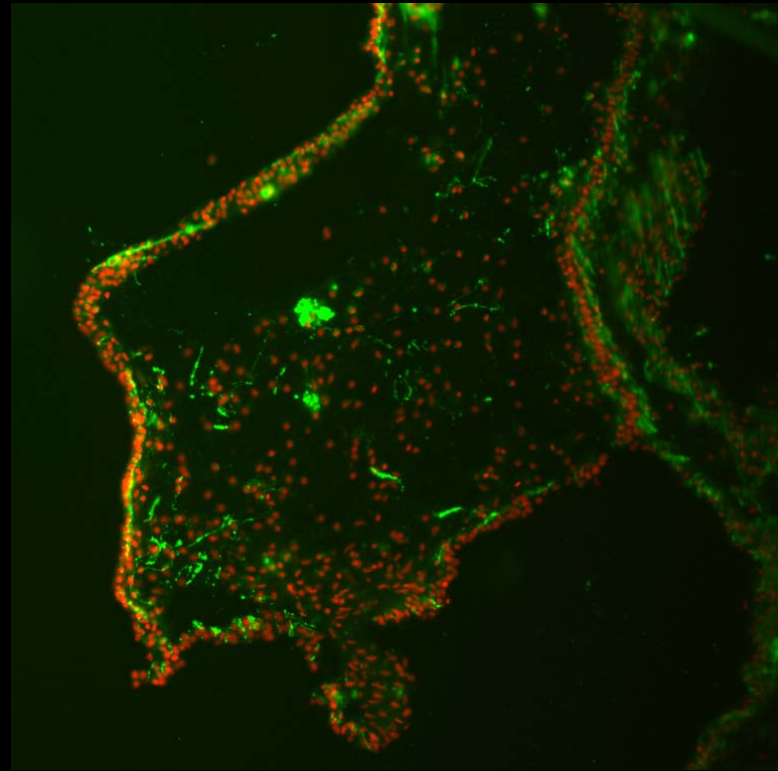


Immunohistochemistry

Control

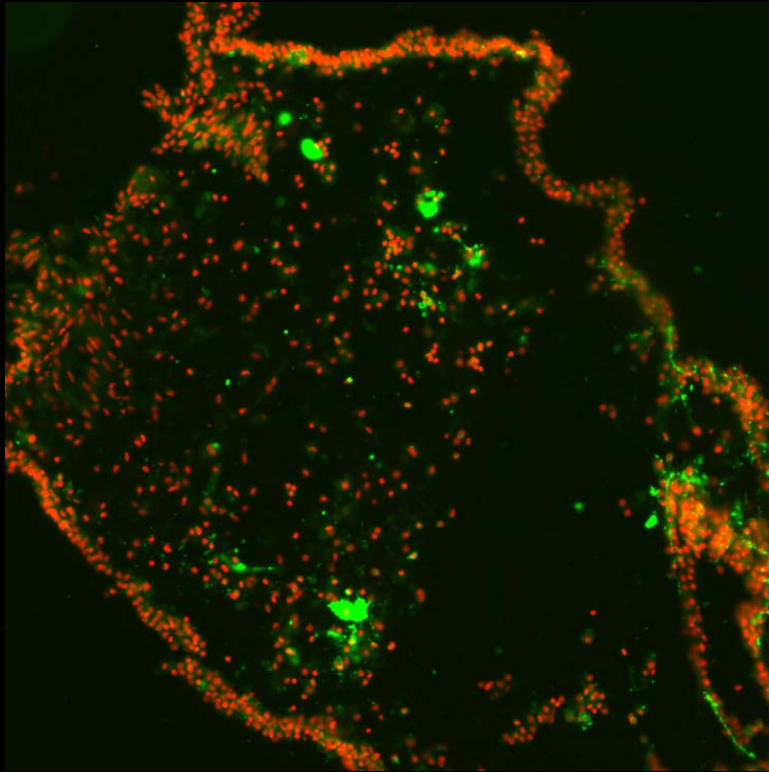


Kanamycin

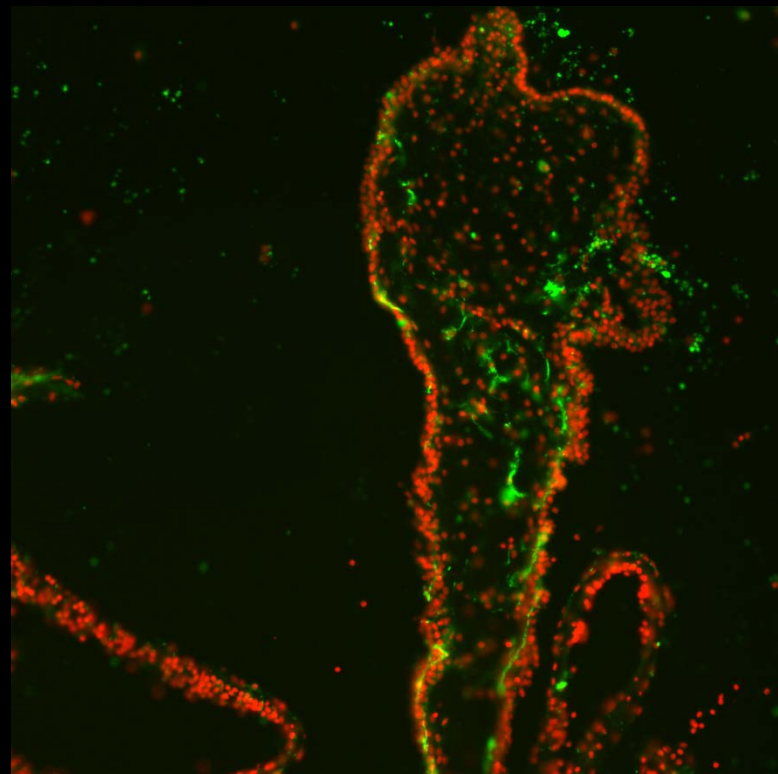


Immunohistochemistry

Control

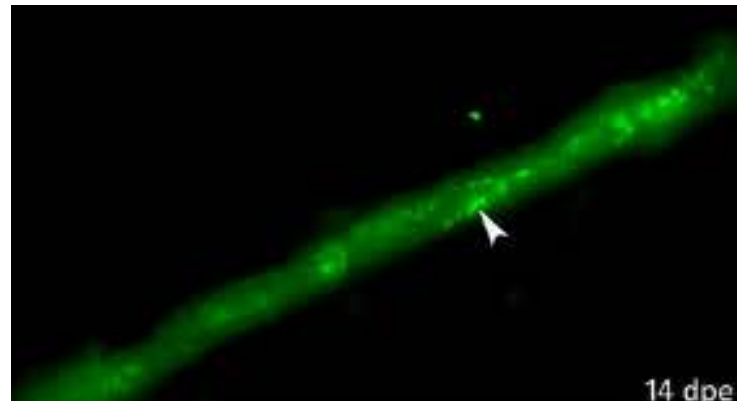


Erythromycin



Observations of RN1 Marker on Previous Experiments

- Less fibers between 5 and 7 dpe and by 10 dpe no nerve fibers or few in rudiment.
- By 14 dpe re-innervation in the rudiment started.



Observations

- Animals treated with Kanamycin and Erythromycin showed more nervous fibers in the blastema.
- Animals in the control group has more neuron- like cells.

Conclusions

- Our results suggest that the intestinal microbiota could be involved in the regenerative process.
- It appears that antibiotics delay both processes: enteric nervous system and tissue regeneration.



Questions