Quantifying Postnatal Regulation of Colostrum in Gilts

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PREPARING UNDERGRADUATES THROUGH MENTORING TOWARD PHD'S

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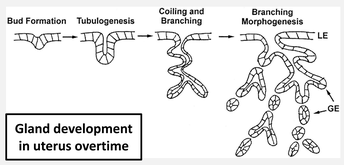
Abstract

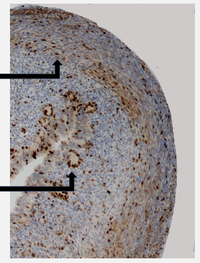
Colostrum is the first form of milk produced by the mammary glands of mammals upon giving birth. In the first 24 hours postpartum, the amount of colostrum consumed by the neonate determines the survival rate and sets the growth trajectory of the infant. Our research aims to develop a novel approach to combine experimental data with quantitative modeling using image-based analysis to describe gilt's growth patterns in the first week postpartum as a function of the amount of colostrum ingested. In this study, we analyze the presence of proliferating versus nonproliferating cells within gilt uterus tissues as an indicator of tissue growth. To achieve this, we developed two MATLAB programs that process gilt uterus tissue histology with Ki67 staining to indicate cell proliferation. The first program segments a uterus by tissue type and produces three new images. Each image contains one of the main tissue types: mucosa, connective, and muscle. The second program uses a color threshold application to calculate the area of proliferating and nonproliferating cells within the sample's respective tissue types. By computing the ratio of proliferating to nonproliferating cells, we seek to determine how cell proliferation is impacted at each tissue type as a result of the amount of colostrum ingested. The findings from this study will enhance our understanding of the impact of colostrum on fertility, and ultimately improve the quality of formula and nutritional supplements administered to infants in the first 24 hours after birth.

Introduction

- Colostrum consumption within the first 24 hours of life is crucial to neonate development and the longevity of piglets.
- Colostrum intake has previously been linked to increased development and efficiency in skeletal muscle tissues, and gastrointestinal functionality.



Gland development in uterus overtime

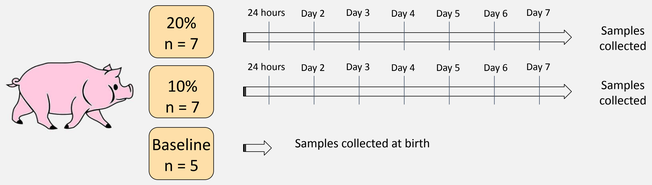


Does a higher intake of colostrum increase cell proliferation within the uterus of the piglet?

Objectives and Methodology

- How will the difference in colostrum intake affect the amount of proliferation in the piglets?
- How will proliferation vary by tissue type?

Ki67 staining makes identifying the proliferation cells much easier, which will make calculating the areas through image analysis ideal



Experimental Procedure:

Feed neonates colostrum:
- ❑ Colostrum 20 group
- ❑ Colostrum 10 group
- ❑ Day 0 group

→ Take histology images on day 7:
- ❑ H&E
- ❑ Ki67

→ Use Image Processing and Code to get proliferating and non proliferating cell areas by tissue type in pixels and then convert to microns.

→ Perform Data Analysis

Digital Image Processing & Mathematical Model

- Cell proliferation is indicated by the presence of brown pigmentation in a Ki67 stained histology sample, while non-proliferating cells are identifiable by blue pigmentation.
- Two MATLAB programs were developed to aid in the analyzation of samples through automation.

Ki67 Tissue Dissection:

- Tissue samples were dissected by hand into three classifications: muscle, connective, and mucosa.

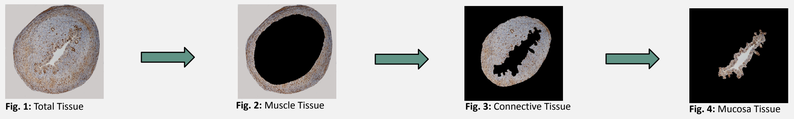


Fig. 1: Total Tissue **Fig. 2:** Muscle Tissue **Fig. 3:** Connective Tissue **Fig. 4:** Mucosa Tissue

Ki67 Proliferation and Nonproliferation Masking:

- The area of cell proliferation in ratio to the area of the tissue sample was identified and calculated in each classification

Fig. 5: Connective Tissue **Fig. 6:** Total Area **Fig. 7:** Proliferation Area **Fig. 8:** Nonproliferation Area

This process is repeated for each tissue sample with the units of measurement in microns

Results

Statistical Analysis

Box Plots

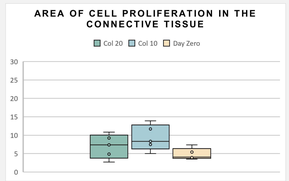


Fig. 9: Boxplot comparing the percentage of connective tissue area covered in cell proliferation by colostrum intake.

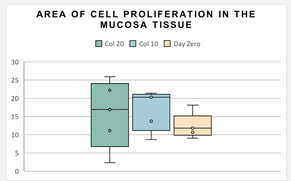


Fig. 10: Boxplot comparing the percentage of mucosa tissue area covered in cell proliferation by colostrum intake.

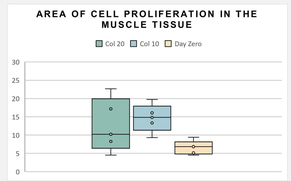


Fig. 11: Boxplot comparing the percentage of muscle tissue area covered in cell proliferation by colostrum intake.

T-Tests

Alpha: 0.05

Connective Tissue	*Col 10*	*Col 20*
Mean	9.363831943	7.009433781
Variance	10.90499455	9.391795378
Observations	7	7
Hypothesized Mean Difference	0	
df	12	
t Stat	1.382659531	
P(T<=t) one-tail	0.095979571	
t Critical one-tail	1.782287556	
P(T<=t) two-tail	**0.191959141**	
t Critical two-tail	2.17881283	

Mucosa Tissue	*Col 10*	*Col 20*
Mean	14.65894028	12.60689816
Variance	10.47028602	42.56372125
Observations	7	7
Hypothesized Mean Difference	0	
df	9	
t Stat	0.745517889	
P(T<=t) one-tail	0.237488902	
t Critical one-tail	1.833112933	
P(T<=t) two-tail	**0.474977805**	
t Critical two-tail	2.262157163	

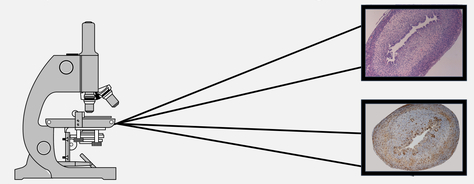
Muscle Tissue	*Col 10*	*Col 20*
Mean	17.0613842	15.99534054
Variance	25.76407149	73.32928403
Observations	7	7
Hypothesized Mean Difference	0	
df	10	
t Stat	0.283335991	
P(T<=t) one-tail	0.39134917	
t Critical one-tail	1.812461123	
P(T<=t) two-tail	**0.78269834**	
t Critical two-tail	2.228138852	

Total Tissue	*Col 10*	*Col 20*
Mean	13.6947188	11.96078758
Variance	11.32182555	34.94626748
Observations	7	7
Hypothesized Mean Difference	0	
df	10	
t Stat	0.674434979	
P(T<=t) one-tail	0.257661478	
t Critical one-tail	1.812461123	
P(T<=t) two-tail	**0.515322957**	
t Critical two-tail	2.228138852	

T-Tests did not yield a significant difference between Colostrum 10 and 20 groups per tissue type

Conclusion

- Box plots show that colostrum 20 results are scattered and sparse. On the other hand, colostrum 10 results are more consistent.
- The median results for colostrum 10 in the boxplots are consistently greater than that of the colostrum 20 median results.
- Day zero piglets in the box plots show the increase in proliferation when compared to the seven day mark.
- T-tests did not pick up a significant difference between colostrum 10 and colostrum 20. However, we can not reject the idea that more colostrum leads to more proliferation and therefore tissue growth.



Future Research

- A random forest and network analysis on our current collection of data to see if our colostrum variables were significantly connected to any other data variables.
- Parameter estimation and nonlinear differential equations to describe growth patterns as a function of the amount of colostrum ingested.
- A higher intake of colostrum may not necessarily imply more cell proliferation, but collecting more data with different colostrum percentages may help us identify the impact of other ranges of colostrum intake on cell proliferation.
- More piglet specimens may be needed to find a statistical significance between the colostrum groups per tissue type.
- Hand collect the area of proliferating and nonproliferating cells in the same sample set to verify accuracy of developed MATLAB automation. This procedure is currently in development.

References/ Acknowledgements

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