Mammary Gland Development and Disease:
Results of a 4-week Research Program with High School Scholars

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BACKGROUND

- We were given the opportunity to participate in a Research Intensive program for four weeks at UMass Amherst by the Great Neck Breast Cancer Coalition. Our goal was to further our knowledge in science and gain experience in the field of research because we are passionate about science and hope to pursue it in the future. Through this amazing experience, we learned about mammary gland development, breast cancer, and why groups like the GNBCC, as well as university researchers, are important for raising awareness and conducting research.

Scientific Objectives

- **Evaluate Mammary Gland (MG) Development-** Mouse MGs develop rapidly in females at puberty, when estrogen is first produced by the ovaries. The defining features of the MG at this time are: terminal end buds (TEB), terminal ends (TE), and ducts. By adulthood, the ducts have filled the fat pad and the TEBs have receded. During pregnancy, alveolar buds begin to form along the ducts. By lactation, these alveolar buds have transformed into lobuloalveolar units. After lactation, the MG undergoes a process of involution where these alveolar structures are removed via processes of apoptosis. Our project used microscopy to evaluate these different stages of development. Our goal was to learn how to identify sensitive windows of development that are important for understanding cancer.
- **Cancer-** The MG undergoes periods of development throughout a female's lifetime. A number of stages of development are considered "sensitive windows" including the embryonic stage, puberty, pregnancy/lactation, and menopause. Understanding these "sensitive windows" is important because the MG is vulnerable to estrogens in the environment during these periods. Lifetime estrogen exposure is a risk factor for breast cancer, and studies of women and their daughters exposed to diethylstilbestrol (DES), a potent pharmaceutical estrogen, revealed links between this estrogen and vaginal cancers as well. To analyze mammary cancer risk, scientists look at the number and size of TEBs and TEs in animals to estimate MG epithelial density, altered stromal composition, and the presence of intraductal hyperplasias (pre-cancerous lesions). Pathologists use histological features to identify cancers in women. Our goal was to understand how these structures can be analyzed in the lab, and learn other techniques. These are summarized on this poster.

ACKNOWLEDGEMENTS

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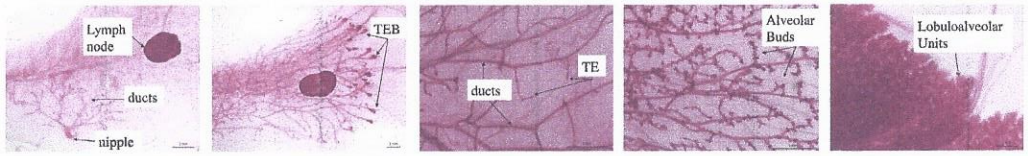
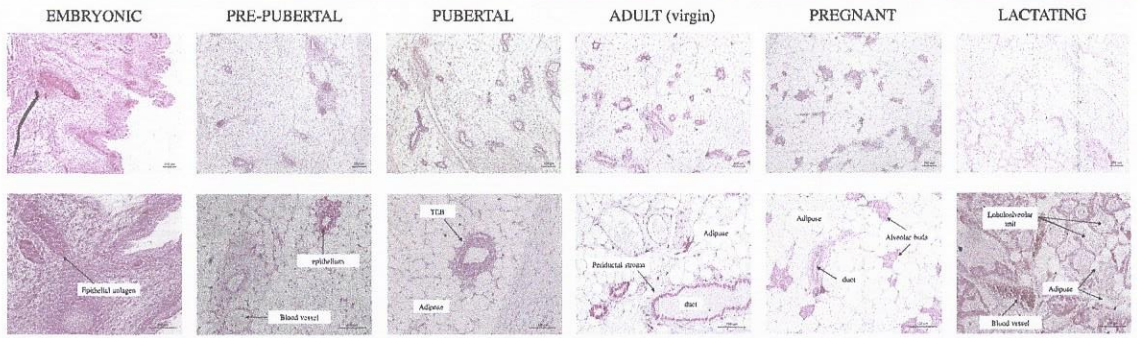
WHOLE MOUNT STAGES

Distinguishing Features



H&E

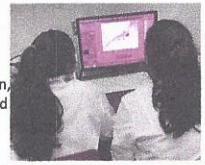


TISSUE PROCESSING

Mammary gland tissues were put into individual cassettes and processed through a series of alcohols. The tissue was embedded in paraffin wax, and set on a cold surface to solidify. A microtome was used to slice the tissues into sections that were 5 µm thick. The sections were collected on slides, which can be analyzed after undergoing H&E, IHC or trichrome staining.

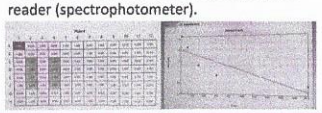
WHOLE MOUNT ANALYSIS

The whole mount MGs were stained with carmine alum and individually bagged in methyl salicylate. To analyze the whole mount MGs, we used a dissection microscope. While studying the structures of the mammary glands, we measured the ductal area, ductal extension, and number and area of TEBs, if present.



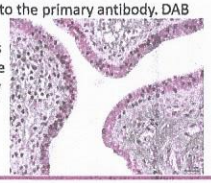
ELISA

The goal of this procedure is to determine the concentration of estrogen present in a blood sample. It is a competition assay, where antibodies are bound to conjugated (tagged) estradiol. Blood serum was added and the estrogen in the sample competed with the conjugated estrogen bound to the antibodies. More estrogen will produce a lower fluorescent signal when measured in a plate reader (spectrophotometer).



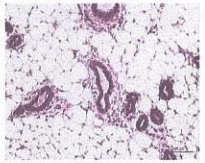
Immunohistochemistry (IHC)

The goal of this procedure is to determine which cells in a tissue express a protein of interest. Here we show our IHC for estrogen receptor (ER)α in the uterus. ERα antibodies bind to the ERα proteins. A secondary antibody binds to the primary antibody. DAB is the chemical which produces a reaction in the cells, ultimately turning them brown. Cells without ERα stain blue.



TRICHROME STAIN

The goal of this technique is to analyze the density of mammary stroma, which is determined by the density of collagen surrounding the ducts. The collagen appears blue on slides after trichrome staining. The darker the blue color, the denser the collagen. In women, more dense breasts are associated with a higher risk of breast cancer.



PCR / GENOTYPING

One project in the lab examines MG development in embryos. It is essential to distinguish male and female embryos. The goal of this technique is to determine whether DNA collected from animals contained the SRY gene. DNA was isolated from embryos. Next, the PCR reaction amplified the SRY gene, if present. Lastly, gel electrophoresis allowed us to visualize the gene of interest.