SCIENCE

Model of Epithelial Cell Changes in PAP-Stained Vaginal Smears throughout the Rat Estrous Cycle: Rat Estrous Model Identification

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Rats are commonly employed for research to observe and understand changes during the reproductive cycle of both rats and humans. The short span and observable cytological changes of the rat estrous cycle make it a predictable component of rat research. Previous studies have divided the cycle into four discrete stages—proestrus, estrus, metestrus, and diestrus—based on the appearance of nucleated epithelial cells, cornified epithelial cells, and leukocytes in vaginal smears. However, the stage of the estrous cycle has mainly been qualitatively determined. In this study, the cycles of four rats were investigated over the course of 21 days with two vaginal swabs a day. The data collected was then stained using a modified Papanicolaou staining protocol, photographed, and classified according to existing evidence for the four stages. Image analysis software was utilized to measure the area of randomly selected epithelial cells present on each photographed sample. Histograms were created, plotting the area of the epithelial cells in $400\ \mu\text{m}^2$ bins against the percent of epithelial cells present in the entire sample for the slide. Findings suggest an interstage that is present when the sample does not have any predominate cell type, which is determined visually and qualitatively as well as quantitatively through the histograms. The methods applied in this study could reduce the confusion and disagreements that exist in published reports by proposing a new model to complement existing approaches for staging the rat estrous cycle, Rat Estrous Model Identification.

Research mentor Nancy Pealez writes: “Gabriella began working on this project with a few other biology students during her freshman year due to discrepancies observed when determining the stage of estrous. Since then Gabriella, along with her team, was able to create a model that more accurately accomplishes this task.”

This figure depicts a shift in the proportion of cells in specific epithelial cell area ranges throughout the course of one four-day estrous cycle. These samples were collected from four rats twice a day through vaginal swabs and were later stained using the modified PAP staining method. The slides were then photographed at 100x magnification and were later analyzed using MIPAR® (Medical Image Processing and Repository) image analysis.