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Cytology and Histology of the Vaginal Epithelium at Different Stages of the Wistar Rat's Oestrous Cycle

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Abstract

Background: The vagina plays a vital role in the female reproductive system and undergoes several histological changes influenced by hormonal variations throughout different life stages and reproductive cycles. There are a few studies that provide detailed accounts of both cytological and histological changes in the vaginal epithelium across the oestrous cycle. **Objective:** This study aimed to examine the microanatomy of the vaginal epithelium of Wistar rats at different stages of the oestrous cycle. **Methodology:** A total of 20 female Wistar rats were randomly assigned to 4 groups, each representing a phase of the oestrous cycle. The rats were maintained under standard laboratory conditions with access to feed and clean water only. The vaginal epithelial cells were collected by gentle saline wash, a smear was made on clean glass slides, the slides were air-dried and fixed in methanol. The slides were stained with Field stains A and B to identify the predominant cell types and determine the phase of the oestrous cycle. The rats were euthanised, and vaginal tissues were harvested and processed for haematoxylin and eosin staining. **Results:** Pro-oestrus phase cytology showed a predominance of basal and intermediate cells. Histology showed a double layer of basal cells and multiple layers of intermediate cells, no cornified cells. Oestrus phase cytology showed abundant cornified epithelial cells. Basal cells and intermediate cells were rarely seen. Histology showed stratified epithelium with a predominance of cells with pyknotic nuclei. Metoestrus/dioestrus cytology showed a predominance of leukocytes, mainly neutrophils, and few intermediate cells. Histology showed a single layer of basal cells, intermediate cells, scant superficial cells, and intraepithelial inflammatory cells. **Conclusion/ Recommendations:** These findings highlight a well-defined pattern of cyclical epithelial growth, differentiation, regression, and immune activity under hormonal control. Further research, such as immunohistochemistry, gene expression profiles as well as comparisons with aged or diseased animal models, is warranted to expand its scope.

Keywords: Microanatomy, Wistar rat, Vaginal epithelium, Oestrous cycle

Introduction

The reproductive cycle in female mammals is regulated by a complex interaction of hormonal signals that induce periodic physiological changes in the reproductive tract. In rodents, this cyclic event is referred to as the oestrous cycle, which plays a fundamental role in preparing the body for fertilisation, implantation, and pregnancy (Cora *et al.,* 2015). The Wistar rat (*Rattus norvegicus*), a commonly used laboratory model, exhibits a regular oestrous cycle. The oestrous cycle in rats and mice averages 4-5 days and is a repetitive, but dynamic, process in which different cell types appear and recede throughout the cycle, reflecting changes in the levels of oestrogen and progesterone secreted by the ovarian follicles. The oestrous cycle is generally divided into four stages: pro-oestrus, oestrus,

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metoestrus and dioestrus (Goldman *et al.,* 2007; Cora *et al.*, 2015; Shreya *et al.*, 2025). However, it can also be divided into three stages: pro-oestrus, oestrus, and metoestrus/dioestrus (Goldman *et al.,* 2007). On cytological evaluation, these stages are defined by the absence, presence, or proportion of cells. The vaginal wall consists of three major histological layers: the mucosa (comprising a stratified squamous epithelium and underlying lamina propria), muscularis, and adventitia (Westwood, 2008). These layers also respond to oestrogen and progesterone levels during the cycle.

Despite the extensive use of Wistar rats in experimental research, few studies provide a detailed account of microanatomical changes in the vaginal epithelium across the full oestrous cycle. Some of the available information is from old journals with black-and-white photomicrographs. Most existing studies emphasise cytological features, leaving a gap in understanding the structural histology of the vaginal epithelium throughout the cycle. The aim of this study was to examine and characterise the microanatomical structure of the vaginal epithelium of female Wistar rats during the different stages of the oestrous cycle using histological and cytological techniques. This study would serve as a reliable reference for researchers and contribute to a deeper understanding of normal reproductive anatomy in rodent models.

Materials and Methods

Twenty (20) female Wistar rats weighing approximately 100-120 grams were procured and allowed to acclimatise for two weeks. The rats were kept in plastic cages with wire-mesh tops to allow airflow. They were provided with food pellets and clean drinking water continuously. Their cages were constantly cleaned. The female Wistar rats were categorised into four groups based on the phase of the oestrous cycle, as determined by vaginal cytology. Each group consisted of five rats, all in one of the four distinct phases of the oestrous cycle. The vaginal cytology protocol was as previously described by Umamageswar *et al.* (2020). Briefly, a sterile syringe was filled with about 0.2ml of normal saline, and the tip was gently inserted into the vaginal orifice of each rat without causing discomfort or injury. The saline was flushed in and out to collect vaginal epithelial cells. A smear was made onto clean glass slides, air-dried, and fixed in methanol (95-100%) for a minute. The slide was stained with Field B for 15 seconds, the edge of the slide was dabbed on a clean cloth to remove excess stain, then stained with Field A stain for 15 seconds. The slide was put under running water to remove excess stain, then air-dried. The stained smears were examined under a light microscope to identify the stage in the oestrous cycle based on the predominant cell type seen.

A midline lower abdominal incision was made to access the pelvic cavity. The vaginal wall was identified as the muscular tubular structure after the cervix and opening to the perineum. The vaginas were collected, fixed in 10% neutral buffered formalin, dehydrated in ascending concentration of alcohol, cleared in xylene, embedded in parafin and processed for Hematoxylin and eosin staining. Slides were examined using a light microscope, and regions of interest were captured with a camera attached to the microscope using Amscope software. White balance correction was performed using Adobe Photoshop. The study protocol was reviewed and approved by the University of Ilorin Ethical Review Committee.

Results

The histology of the vaginal wall was composed of the epithelium (mucosa), subepithelium (submucosa), muscular layer and the adventitia layer. Epithelial ridges and subepithelial papillae were seen (Figure 1). The subepithelium was fibrocollagenous, containing fibroblasts with spindle tapered-end nuclei surrounded by eosinophilic (pinkish) collagen. Also seen were vascular channels. The muscular layer was well defined. The adventitia was composed of loose fibrous connective tissue and vascular channels. From Figure 2, the cytology of the pro-oestrus

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phase showed predominance of nucleated epithelial cells, mainly basal and intermediate cells. The basal cells were smaller than the intermediate cells, with more darkly stained nuclei and little to no cytoplasm seen. The intermediate cells showed fairly basophilic cytoplasm. The histology showed stratified epithelium: a double layer of basal cells and multiple layers of intermediate cells. There were no cornified cells seen. The cytology of the oestrus phase in Figure 3 showed abundant cornified epithelial cells with pyknotic or no nuclei. Cornified epithelial cells had a lower nuclear-to-cytoplasmic ratio than intermediate cells. The cytoplasm in the cornified cells was more, and the nucleus was smaller. Basal cells and intermediate cells were rarely seen. The histology showed stratified epithelium with predominance of cells with pyknotic nuclei or loss of nucleus. The cytology of the metoestrus/dioestrus showed predominance of leukocytes mainly neutrophils (Figure 4). The neutrophils had darkly stained multilobulated nuclei (with two to three lobes). Few intermediate cells were seen. The histology showed a single layer of basal cells, intermediate cells and scant superficial cells. Neutrophils were seen with the epithelium as well as in the lumen. Red blood cells were also seen within the lumen.

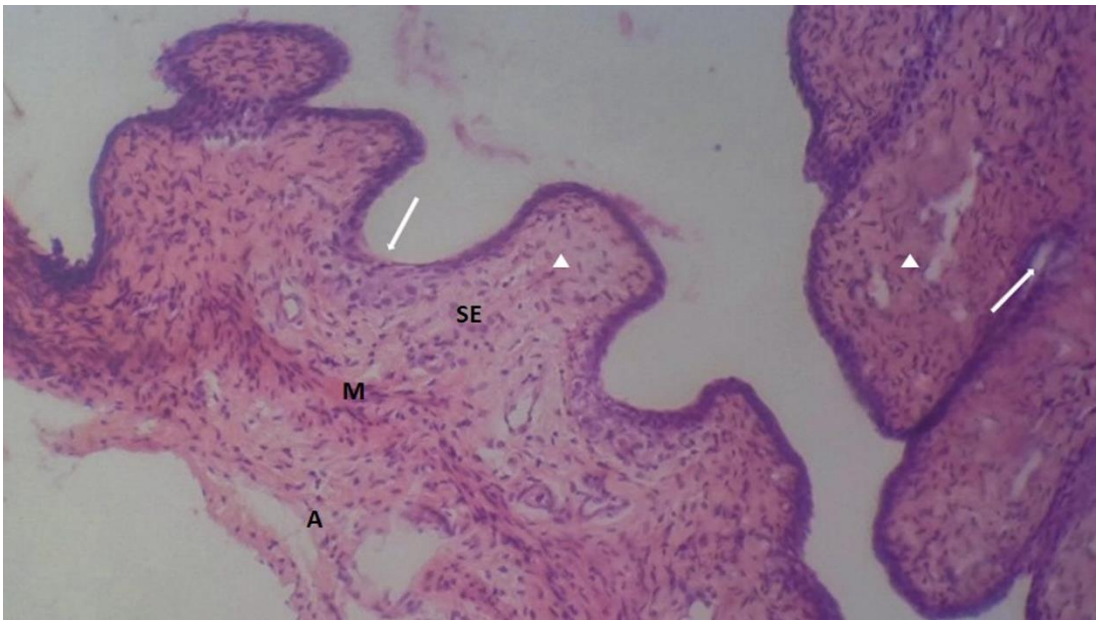

Figure 1: Photomicrograph of the vaginal wall section from the pro-oestrus group (H&E, 4x magnification) demonstrating thin epithelium, thick fibrocollagenous subepithelium (SE), muscular layer (M) and adventitia (A). Arrow: epithelial ridges, arrowhead: subepithelial papillae.

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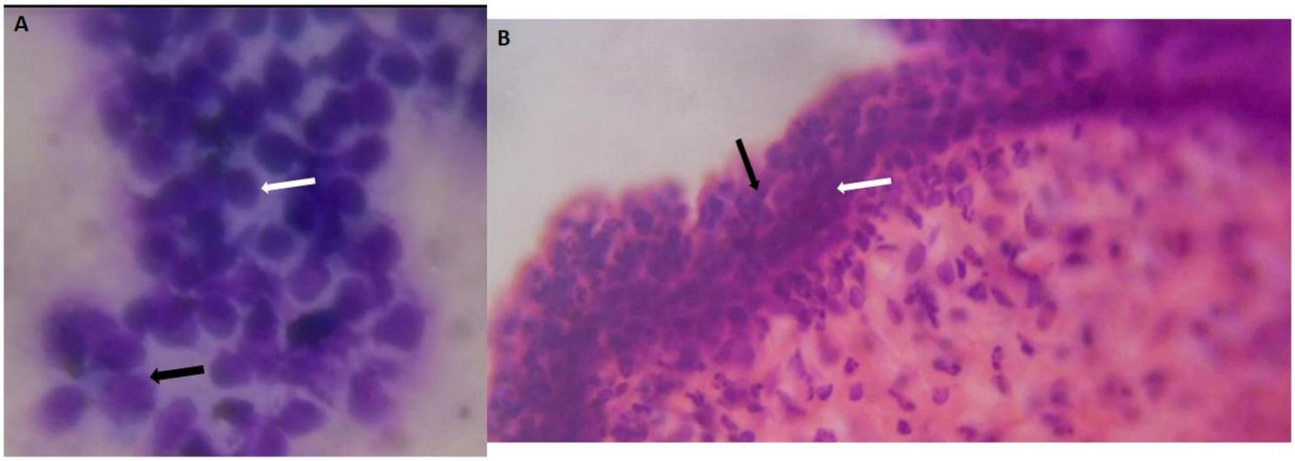

Figure 2. Photomicrograph of a vaginal section from the pro-oestrus group. A: Cytology (Field solution A&B, 400x magnification) demonstrating basal (white arrow) and intermediate cells (black arrow). B: Histology (H&E, 400x magnification) showing the epithelium with double layers of basal cells (white arrow) and multiple layers of intermediate cells (black arrow).

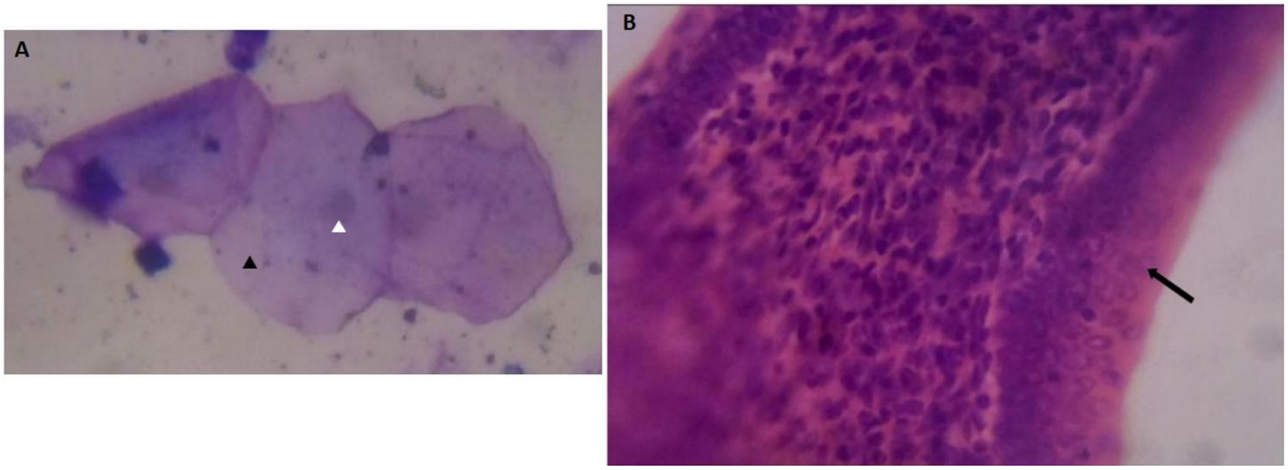

Figure 3: Photomicrograph of a vaginal section from the oestrus group. A: Cytology (Field solution A&B, 400x magnification) demonstrating superficial cells (Cytoplasm: black arrowhead, fading nucleus: white arrowhead). B: Histology (H&E, 400x magnification) demonstrating multiple layer of superficial cells (black arrow).

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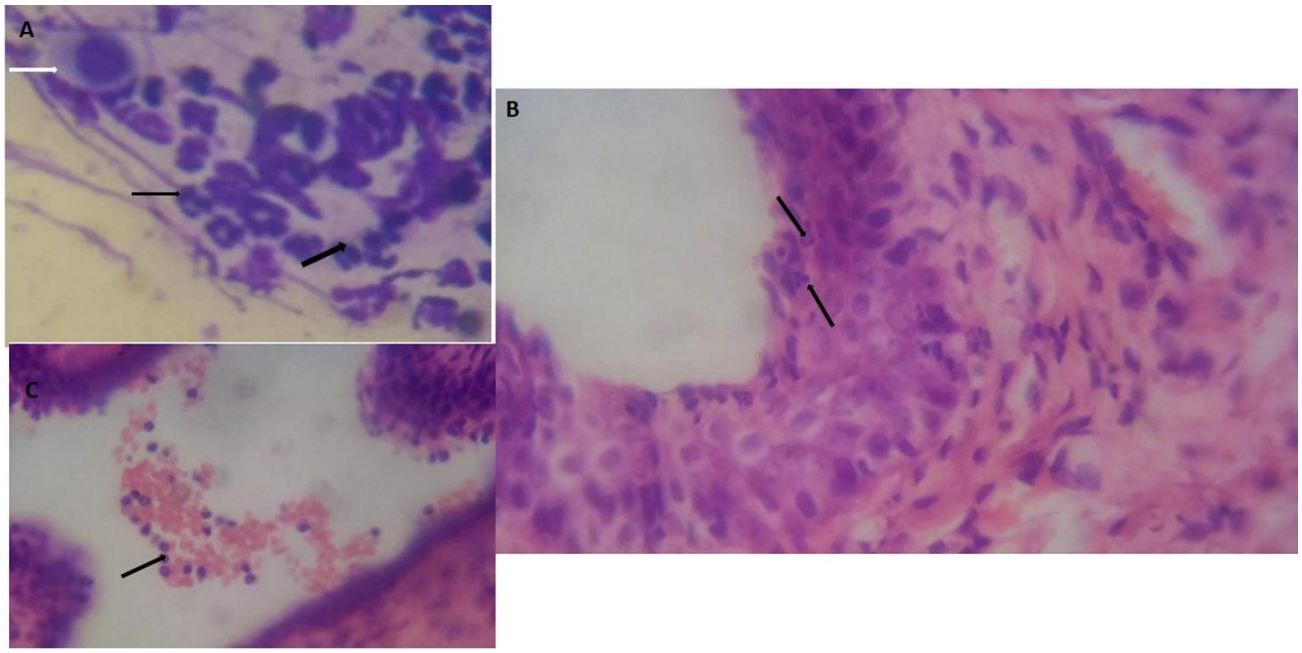

Figure 4: Photomicrograph of a vaginal section from the metoestrus/dioestrus group. A: Cytology (Field solution A&B, 400x magnification) demonstrating intermediate cells (white arrow) and neutrophils (black arrow) B: Histology (H&E, 400x magnification) demonstrating intraepithelial neutrophils (black arrow), intermediate cells, and a single layer of basal cells. C: Histology (H&E, 40x magnification) demonstrating neutrophils (black arrow) and red blood cells within the lumen.

Table 1: Cytology and histology by oestrous phase

Oestrous Phase	Cytological Findings	Histological Findings
Pro-oestrus	Predominance of basal and intermediate cells.	Stratified epithelium consisting of a double layer of basal cells and multiple layers of intermediate cells. No cornified cells present.
Oestrus	Abundant cornified epithelial cells with pyknotic nuclei. Basal and intermediate cells are rarely seen.	Stratified epithelium showing a predominance of cells with pyknotic nuclei.
Metoestrus / Dioestrus	Predominance of leukocytes, specifically neutrophils. Few intermediate cells are present.	Epithelium consists of a single layer of basal cells, intermediate cells, and a scant superficial layer of cells. Neutrophils are observed within the epithelium and the lumen.

Discussion

Histological evaluation of the vaginal epithelium across the oestrous cycle in Wistar rats reveals a hormone-dependent series of morphological transformations that reflect fluctuations in oestrogen and progesterone levels. Each phase,

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pro-oestrus, oestrus, metoestrus/dioestrus, presents unique structural features that serve distinct physiological roles in the reproductive cycle. The identification of squamous epithelial cells with prominent basal and intermediate cells in the pro-oestrus phase correlates with findings by previous studies (Umamageswar *et al.*, 2020; VK *et al.*, 2025; Ajayi *et al.*, 2025). Oestrogen levels begin to rise during pro-oestrus, stimulating epithelial proliferation and basal mitotic activity (Hasbi Hasbi & Gustina, 2020; Ajayi *et al.*, 2025). The pro-oestrus phase corresponds to the follicular phase in the human menstrual cycle. Leukocytes are typically absent in pro-oestrus.

The oestrus phase shows superficial anucleated cornified cells with pyknotic nuclei and few intermediate and basal cells. The superficial cells have undergone full keratinisation, losing their nuclei in the superficial layer. This supports prior studies that describe oestrus as the phase of maximal epithelial cornification, driven by peak oestrogen levels (Marcondes *et al.,* 2002; Westwood, 2008). The keratinised stratified squamous epithelium observed in the oestrus phase in this study aligns with the oestrogen-driven cornification process noted by Westwood (2008) and Goldman *et al.* (2007). While pro-oestrus represents the buildup of the mucosal lining under oestrogenic stimulation, oestrus marks its function as a protective interface during copulation (Westwood, 2008). The absence of leukocytes is consistent with observations by Hubscher *et al.* (2005), who documented a largely sterile lumen during this phase. The oestrus phase corresponds to ovulation in humans (Ajayi *et al.,* 2025).

Histological analysis during metoestrus/dioestrus revealed prominent leukocytic infiltration, presence of few to no cornified cells and a single basal layer. These findings are in line with previous studies (Marcondes *et al.,* 2002; Hubscher *et al.,* 2005; Umamageswar *et al.,* 2020; VK *et al.,* 2025*)* that emphasised the immune reconstitution role of this phase. The reduction in the basal cell layer also supports Westwood's (2008) description of hormonal withdrawal and epithelial regression during dioestrus. In metoestrus/dioestrus, the vaginal epithelium begins to regress, showing partial desquamation of the cornified layer and a reduction in thickness. The cytology showed prominent neutrophils with few nucleated epithelial cells, unlike the sterile luminal environment of pro-oestrus and oestrus. This phase serves as a transitional period, reflecting the withdrawal of oestrogen and the early effects of rising progesterone (Marcondes *et al.,* 2002; Ajayi *et al.*, 2025). Metoestrus and dioestrus represent the early and late secretory phases in humans (Ajayi *et al.*, 2025).

Conclusion

The histology and cytology of the Wistar rat vaginal epithelium across the oestrous cycle reveal a well-defined pattern of cyclical epithelial growth, differentiation, regression, and immune activity under hormonal control. This study contributes to the foundational knowledge required to understand and interpret experimental and clinical research in female reproductive biology.

Recommendations: This study provides valuable baseline data, however, further research is warranted to expand its scope. Immunohistochemistry could be employed to identify epithelial cellular markers for differentiation and proliferation, such as cytokeratin and Ki-67, as well as specific immune cell populations. Investigating the gene expression profiles of vaginal tissues across the oestrous cycle would also offer deeper insight into molecular regulation. Future studies should also incorporate quantitative morphometry and comparisons with aged or diseased animal models to broaden the relevance of the findings in clinical settings such as menopause or vaginal atrophy.

Scientific implications of the study: This study provides histology and cytology images of the vagina epithelium, which serve as a reference for rodent reproductive studies and provide a

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baseline for evaluating the toxicological effects of drugs on the reproductive system.

Limitations to the study: This study acknowledges limitations. The research is limited to Wistar rats maintained under controlled laboratory conditions, which may not reflect the full range of reproductive variability found in other rat strains or the wild population. The study emphasises only morphological features observable via routine cytological and histological staining.

Ethical approval: This study protocol was approved by the University of Ilorin Ethical Review Committee (Approval number: UERC/ASN/2024/3012)

Conflict of interest: The authors have no conflict of interest to declare.

Acknowledgement: Not applicable

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