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Vaginal Lavage and Cytology

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We use this protocol and it's working.

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Disclaimer

The authors declare no competing interests or conflicts.

Abstract

This protocol details the procedure for identifying the phase of the estrous cycle in female rats using vaginal cytology. Cells obtained from vaginal lavage are fixed, stained, then visualized. The vaginal lavage method provides a non-invasive and less stressful way to collect samples from the animal, while offering reliability comparable to the vaginal smear method.

Materials

Fisherbrand Superfrost Plus Microscope Slides Precleaned (Cat no: 1255015; Fisher Scientific)
Hydrophobic pen or crayon
Milli-Q water
Disposable Graduated Transfer Pipettes (Cat no: 137119CM; Fisher Scientific)
Slide book
Slide storage container
Hema 3 Manual Staining System and Stat Pack (Cat no: 23-123869; Fisher Scientific)
BZ-X800 Keyence Microscope
Microscope Slides Staining Jars (4) and Rack (1)
Timer
Paper towels

Safety warnings

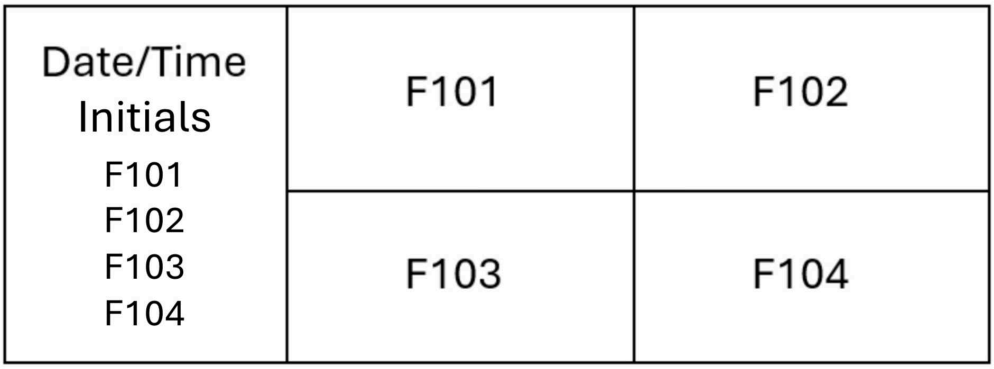
The HEMA 3 Fixative solution is flammable and must be stored in a flammable cabinet. Avoid leaving slides in the third Hema stain for too long, as prolonged exposure to the purple dye can hinder visualization.

Ethics statement

All procedures involved were conducted per the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, San Diego.

Preparation for Sample Collection

1 Prepare precleaned and frosted microscope slides and label with a hydrophobic pen or crayon as shown below.



Example slide for sample collection

2 Prepare a slide book that will contain all slides containing vaginal lavage samples.

3 Before starting sample collection, organize and arrange the labeled slides in the slide book. Keep the slides in the book and leave it open to facilitate easy placement of samples onto the slides.

Sample Collection

1d

4 Using **Disposable Graduated Transfer Pipette Tips**, take up a small amount of MilliQ water (< 1mL) to be used for the vaginal lavage.

5 Restrain the female rat and the pipette in a position where the pipette is directly above and parallel to the rat's vaginal cavity. Make sure to hold the rat firmly by restraining it between your nondominant hand and abdomen. This will prevent it from squirming, which may make it difficult to collect samples.

6 If the rat is about to urinate or defecate, wait for it to stop. Urine will contaminate the sample and will make the sample nonviable for visualization.

7 Using the same aliquot of MilliQ water (~ 50 - 75 µL), gently flush the vaginal cavity three times to collect the sample. Make sure not to insert the pipette tip beyond the vaginal opening.

8 Dispense the sample onto the appropriate location on the prelabeled slide. Dispense in a constant velocity to minimize bubble formation, which can obstruct microscopy visualization. While dispensing, strive to make a thin layer to minimize drying time.

9 After collecting all samples, carefully transport and place the slide book on a flat surface to allow slide samples to dry at room temperature overnight. Transfer dried slides from the slide book to a slide storage container. Samples can now be stored and stained. 1d

Preparation for Staining Using HEMA 3 Fixative

10 Clear out a space to stain slides in the fume hood.

11 Place two strips of paper towels on the counter. One strip will be used to soak excess liquid in between stains. The other strip will be used to dry slides in the fume hood.

12 Load dried slides on a clean Microscope Slides Staining Rack.

13 Prepare four clean Microscope Slide Staining containers to be filled with HEMA 3 stains or MilliQ water. Fill each container separately with the following solutions from the HEMA 3 kit: Fixative, Solution I, Solution II. Fill an additional container with MilliQ water.

HEMA 3 Staining 1d 0h 1m 15s

14 Begin by dipping the loaded slide rack in the HEMA 3 Fixative container for 30 seconds. Gently tap the rack on the paper towel to absorb excess liquid. For steps 14 - 16, try to absorb as much excess solution as possible to avoid contamination between solutions. 30s

15 Dip slide rack in the HEMA 3 Solution I container for 30 seconds. Gently tap the rack on the paper towel to soak excess liquid. 30s

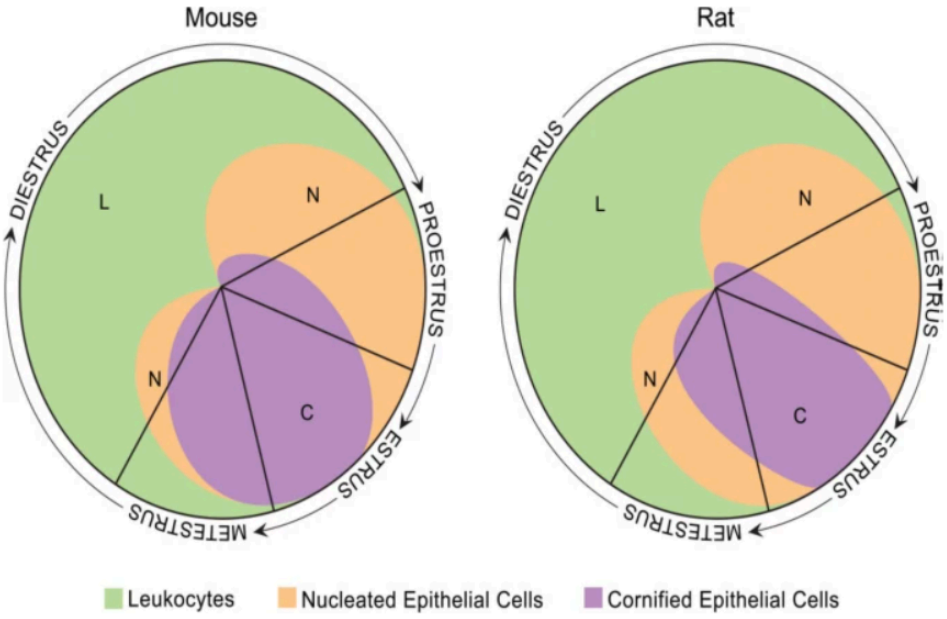
16 Dip slide rack in the HEMA 3 Solution II container for 15 seconds. Avoid leaving slides in this third Hema stain for too long, as prolonged exposure to this dye can hinder visualization. Gently tap the rack on a paper towel to absorb excess liquid. 15s

17 Dip slide rack thoroughly in MilliQ water jar to remove all excess dye.

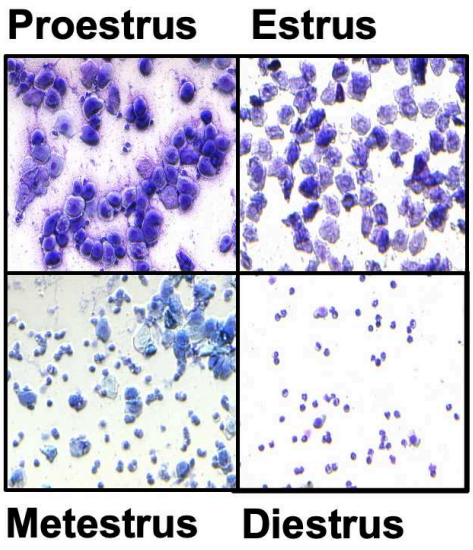
18 Transfer stained slides to paper towel and leave in fume hood to dry overnight. Return stained and dried slides back to slide storage container. 1d

Microscopy

19 Using a **BZ-X800 Keyence Microscope** (Keyence, Itasca, IL) or an analogous microscopic visualization method, identify estrous phase based on cell type distribution.



Distribution of cell types across estrous phases. Figure obtained from (Ajayi and Akhigbe, 2020).



Cell types found in estrous phases. Figure obtained from (Sneddon et al., under review).

Protocol references

Ajayi, A.F., Akhigbe, R.E. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertil Res and Pract 6, 5 (2020). https://doi.org/10.1186/s40738-020-00074-3

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