

Mouse Vaginal Cytology (Smearing) Protocol

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When beginning to smear the mice, it is important to remember to establish an appropriate labeling system to track each mouse's cycle individually. Mice should be housed no more than 3 females per cage and generally, they are ear punched for identification. One mouse will have no ear punch, one will be left ear punched and one will be right ear punched. It is important to set aside a time in the morning each day that you will smear these females (generally around 10am). All hormonal surges occur in the afternoon in mice, so you want to keep it as consistent as possible and be sure to always smear before 3pm.

- 1) To begin, you must make a saline solution, which will be used to perform the vaginal lavages on the mice. The recipe for this solution is as follows:

Saline Solution

9g NaCl

1 Liter Sterile Water

Combine ingredients in a sterile, autoclaved 1L bottle and invert repeatedly to mix the solution.

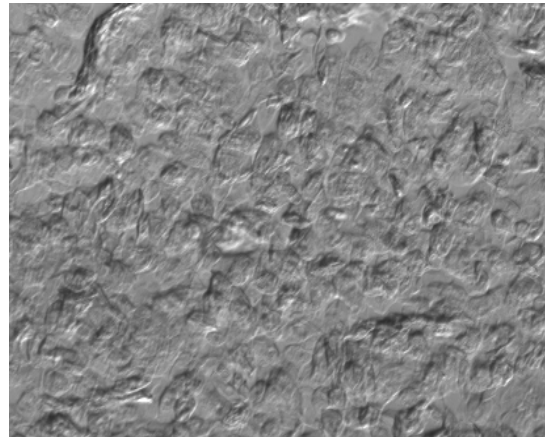
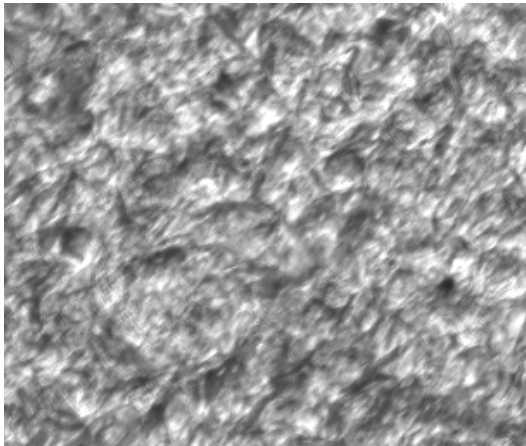
- 2) Pour some of this saline solution into a 200mL beaker and bring another beaker or plastic container with as a means to dispose of cellular debris and rinses.
- 3) Obtain a 48-well plastic plate from the follicle culture room and write the labeling system on top of each well in permanent marker.
For example, a mouse from cage 1 and no ear punch would be 1-0; a mouse from cage 1 with a right ear punch would be 1-R, etc.
- 4) Be sure to bring your beaker of saline solution, empty beaker, labeled plate, and an eyedropper downstairs with you.
- 5) Once downstairs, open the first cage of animal and remove your first mouse. (It is helpful if you put the wire rack back onto the cage so that the animal can grip it while you are smearing her.)
- 6) Place the animal onto the wire rack (I find it is helpful to put their head in the direction of the food).
- 7) Fill up the eyedropper about $\frac{3}{4}$'s of the way with the saline solution and keep it in the beaker of saline filled
- 8) Grasp the base of the tail with your index finger and thumb with your non-dominant hand, and use your remaining 3 fingers to put pressure onto the back of the animal and arch its butt in the air towards you. When doing this you will keep the animal relatively immobile, which will allow you to properly smear the animal.

- 9) Pick up your eyedropper and insert it into the vagina of the mouse, flush the saline into the vagina and suck it back into the eyedropper
- 10) Pull the eyedropper out of the mouse and expel the cells and saline from the eyedropper into the corresponding labeled well on your plate
- 11) Put the mouse back into the cage and remove the next mouse. Repeat until you have smeared all of your mice.

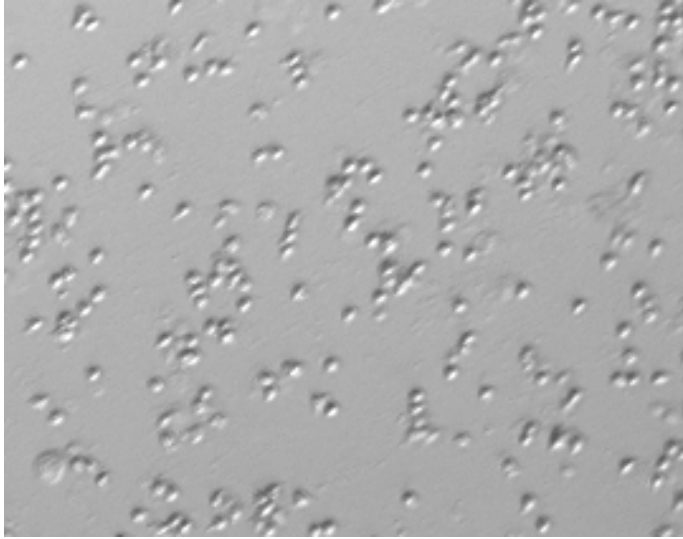
To Read the Cells from Vaginal Cytology...

You will need to view the cells until a 10X objective on the microscope and record the cell types for each individual mouse. There are 4 stages of the mouse cycle: Estrus, Proestrus, Metestrus, and Diestrus. The following are descriptions and images of the different cycle stages:

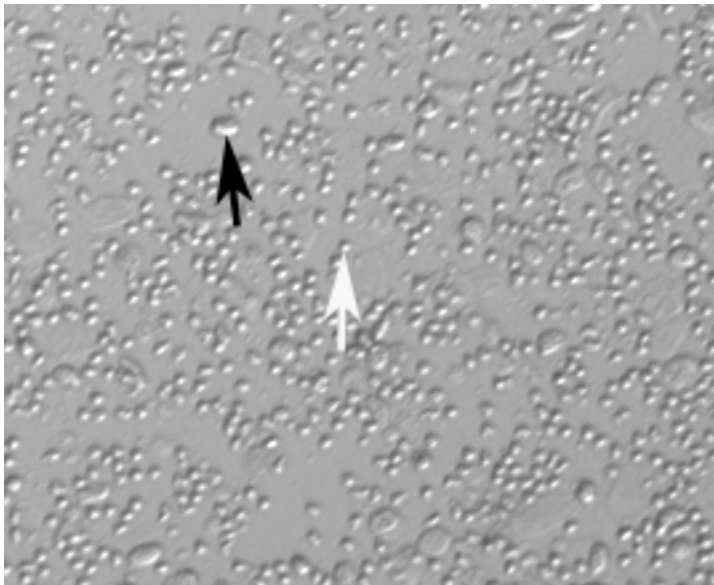
Estrus: cornified epithelial cells are present in the sample. This cycle stage persists for about 36 hours. These cells can be marked as C in your records.



Metestrus: follows estrus and is a leukocyte containing sample. You may also see a few remaining cornified cells in this stage. This cycle stage lasts for about 24hrs. These cells can be marked as L in your records.

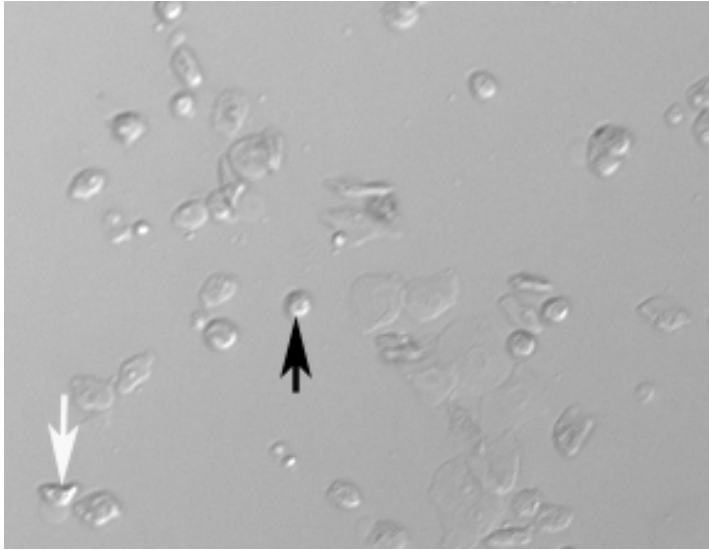


Diestrus: follows Metestrus and is also a leukocyte containing sample. You will begin to see some larger, more rounded nucleated cells. This cycle stage lasts for around 24hrs. These cells can be marked as L in your records.



In this sample, you see a cell turning from a leukocyte white arrow to a larger, more rounded nucleated cell (black arrow)

Proestrus: follows Diestrus and contains nucleated cells. These cells will be sparse in the sample and usually it is hard to catch a true proestrus phase of the cycle because it persists for only 12hrs. These cells can be marked as N in your records.



In this sample you can see that the nucleated cells (black arrow) are already beginning to transition into the cornified cells of estrus (white arrow)

A few things to note...

When keeping records of animal cycling, cycle stages will not always be perfect. You may observe an extra day of Leukocyte cells, or perhaps a lengthened period of estrus. Most of the time, you will observe a normal cycle which goes L, L, N, C, L, L, N, C, etc. depending on which cycle stage you observe first.

If you are trying to observe animals that are in Metestrus or Diestrus, you will most likely not be able to tell a difference with certainty just by looking at the samples. You must track the cycle carefully and note that Metestrus will occur just after Estrus and then Diestrus will be the following day.

If the cells are transitioning between cycle stages and there are 50% of each cell type, or 60/40 (ie- Cornified and Leukocytes), they can be marked as C-L because it will not be a completely clear read.

Again, one of the most important things is to pick a time at which you will smear the animals and smear them at this time everyday for consistency. LH surges occur in the afternoon, usually around 3-4pm, so it is important to smear mice before 4pm and never after.

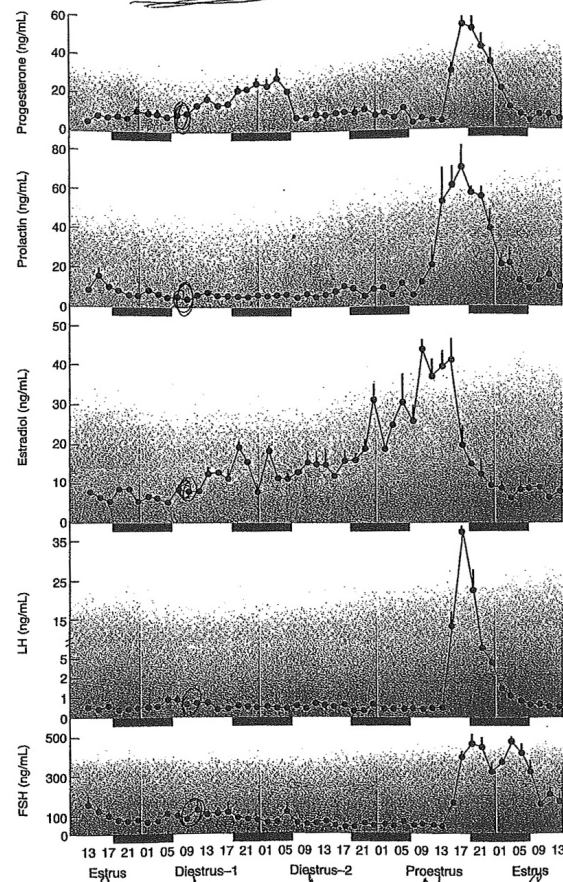


Figure 9.10 Hormonal profiles throughout the 4-day estrous cycle in the female rat. The horizontal red bars and white vertical lines indicate the dark period (18:00-6:00) and midnight. Values are means \pm SEM. LH, luteinizing hormone; FSH, follicle-stimulating hormone. Adapted from Smith *et al.* (1975).

lots of
15 min