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Collection – Staining– Analysis of vaginal smears

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We use this protocol and it's working

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Abstract

The 4 stages of the rodent's female cycle are:

1. Metestrous – M
2. Diestrous -D
3. Proestrous -P
4. Estrous – E

Transitional stages, i.e. showing characteristics of two consecutive stages e.g. M-D or D-P, are not uncommon, particularly if smears are taken very early or late in the day.

The most commonly used smearing techniques (methods of obtaining vaginal cell samples) are:
a) Lavage or washing with saline or water from a pipette.
b) Swab or cotton bud (moistened with saline or water).

The standard swabbing method consists of inserting a moistened cotton bud swab into vagina, gently removing the cells from the vaginal lumen and walls and transferring the cells to a glass slide. It is the quickest method of smearing and the smears retain their original appearance indefinitely. However, because there is a risk of causing pseudopregnancy if the cervix is stimulated during estrus, we collect samples without inserting the cotton swab into the vagina.

Guidelines

These guidelines are designed for females that are housed at a 12:12 hours standard cycle, with a light: dark cycle of 12:12 and lights on at 06:00 GMT.

Materials

STAINING:

Reagents:

Fisher HealthCare Hema 3 Manual Staining System and Stat Pack

1. Hema 3 Fixative Solution - containing Methanol– light blue color
2. Hema 3 Solution I - containing 0.01% Na Azide - red color
3. Hema 3 Solution II - containing 0.01% Na Azide - dark blue color
4. Deionized Distilled water
5. Coplin jars to hold each solution

Storage and Stability:

- Store at room temperature (15 °C - 30 °C)
- Change stain weekly. Keep original bottles and Coplin jars tightly closed.
- Do not use after the expiration date.
- The presence of excessive precipitation in the stain solution or on the stained slides and inadequate differentiation of cell types may indicate deterioration.

Sample collection

1 Pre-label slides with study number, animal numbers, date and time of collection, using a permanent marker, and place them on a suitable tray. Slides can be set up on a date basis, with one set of slides for all females each day.

2 Hold the mouse by the base of the tail, lifting the back a little.

3 Moisten the cotton wool tip slightly by dipping into a jar of saline or distilled water and sharply flick off any surplus.

4 Collect sample with a rotating action of the swab and at an angle of about 45o to the animal's body. The rotating action of the swab stick is continued, in the same direction.

5 With the swab held almost horizontally, to ensure cells from the full length of the vagina are transferred, the tip is rolled gently onto a clean, pre-labelled glass slide, below the relevant animal number.

6 It is important to avoid getting too much fluid on the slide (usually resulting from too much pressure being applied when rolling the swab onto the slide) and to avoid urine contamination as these factors can mask the cells and make reading very difficult.

7 The swab stick is discarded, a new one being used for each animal.

8 Let the slides dry completely (it should take 5-10 minutes) on a horizontal position before proceeding with staining.

Staining

9 Transfer each solution into a Coplin jar.

10 Dip dry smear/slide 5-10x for one second each in Hema 3 Fixative Solution.

11 Cover Fixative when not in use to avoid MethOH evaporation.

12 Allow excess to drain/blot slides.

13 Dip slide 5-10x for one second each in Hema 3 Solution I.

14 Allow excess to drain/blot slides.

15 Dip slide 5-10x for one second each in Hema 3 Solution II.

16 Allow excess to drain/blot slides.

17 Rinse slide with DI water.

18 10. Allow excess to drain.

19 11. Allow to dry.

20 **NOTE**
Staining intensity may be varied by increasing or decreasing the number of dips in Solutions I and II:
Eosinophilic staining may be intensified by increasing dips in Solution I.
Basophilic staining may be intensified by increasing dips in Solution II.

Analysis

21 The normal 4-5-day cycle will be in the sequence M, D, P, E, M, D, P, E etc...

21.1 - Single stage (E, M, D or P) - if within the normal range of appearance.

21.2 - Transitional stage (e.g. MD or DP) - if characteristic of both stages.

22 These stages of the estrous cycle can be recognized by the presence, absence or proportional numbers of epithelial cells (two types), cornified (keratinized) cells and leucocytes. It is the balance of proportions between these cell types that permits the classification of the stage of the cycle between successive ovulations. Occasionally mucus is also seen, especially in acyclic females.

TABLE 1. Vaginal smear cell types and numbers during the oestrous cycle of the rat[1]

Stage	Typical cell numbers					Reliability*
	Leucocytes	Nucleated epithelials	Cornified	Non-nucleated epithelials	Total	
Oestrus	-	-/+	+++	+/-	+++	+++
Metoestrus	+++	-	-/+	+	+++	++
Di-oestrus	++	+/-	+/-	+/-	+/++	+++
Pro-oestrus	-	++	-/+	-	+/++	++

vaginal cytology.pdf

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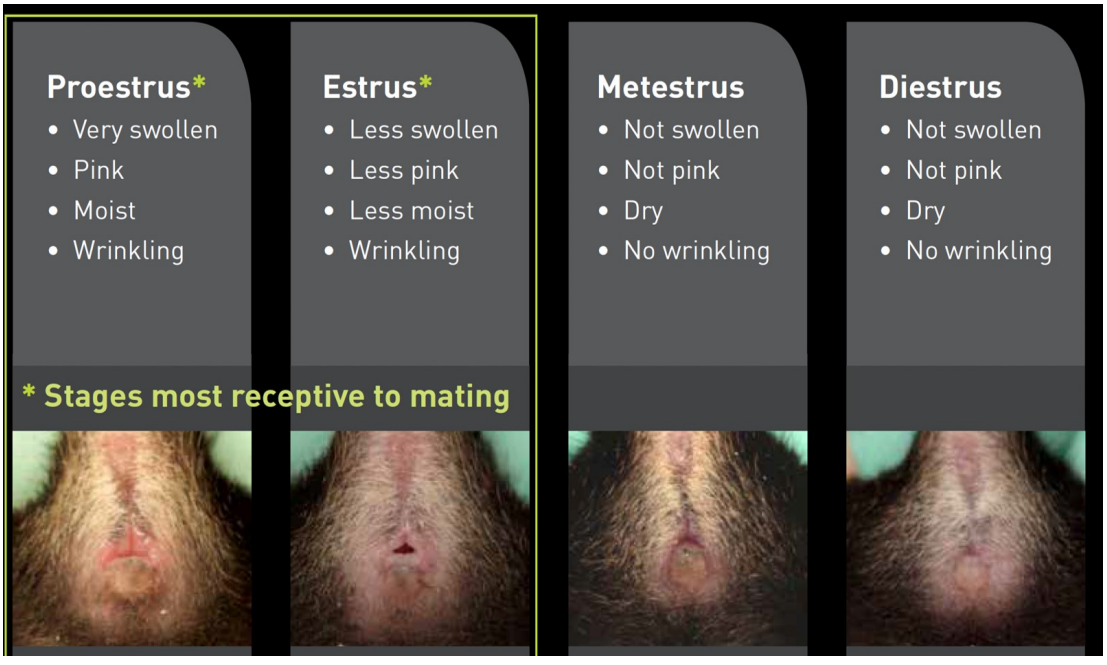

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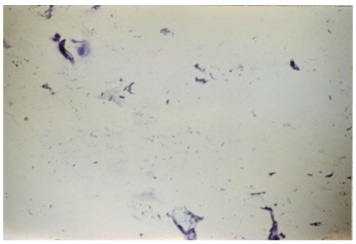
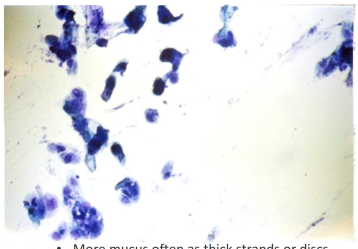
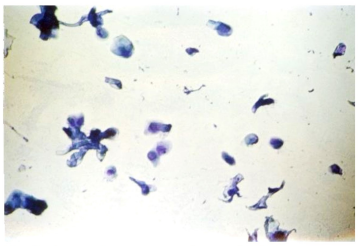
http://oslovet.veths.no/teaching/rat/oestrus



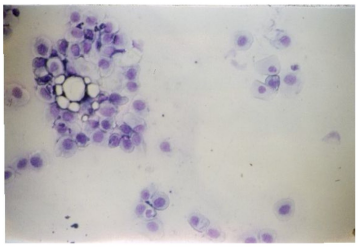
D
- traces of secretory material with cellular debris.
- Few parabasal and intermediary cells
- Few L in swab smears / predominantly L in vaginal lavage



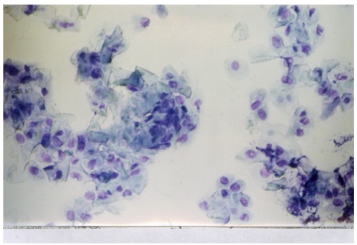
D→P
- More mucus often as thick strands or discs
- parabasal irregular or shrunken cells
- some intermediary cells
- Fewer L



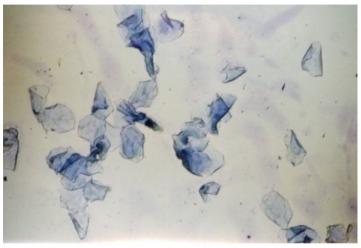
Early P
- Less mucus and some debris
- More parabasal and intermediary cells L are rare



P



P → E



E
- Only Keratinized superficial cells
- Few intermediary cells with nucleus
- No L

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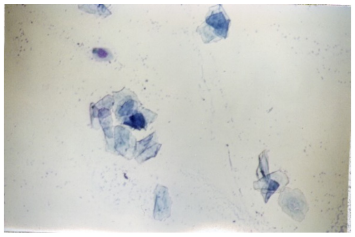
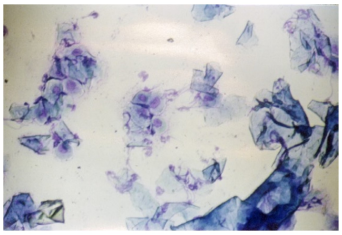
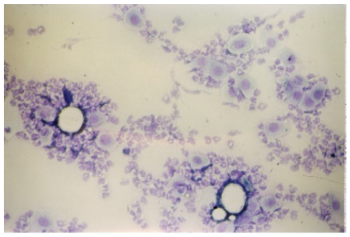


E cont.
- Few intermediary cells with nucleus
- Mainly Keratinized cells
- No L

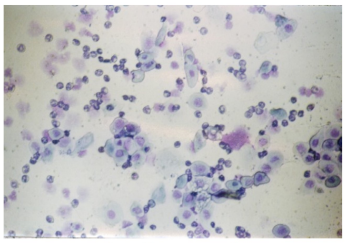


E → M
- Keratinized superficial cells
- Presence of L
- few intermediary cells with nucleus

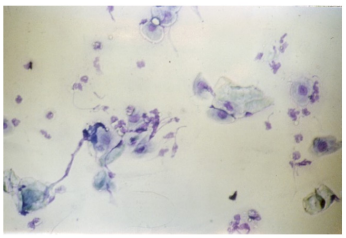


M

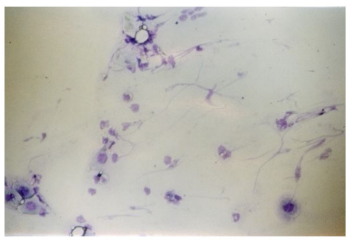
All 3 types of cells (K, EpN, and L):
- Dominated by large number of L
- Some Cornified cells
- Some intermediary cells



M
- Dominated by presence of L
- Few cornified cells
- Few intermediary cells
- Few parabasal cells and larger int. cells



M → D
- Reduction in cell number
- Reappearance of mucus (thin strands)



M → D
- Reduction in cell number
- Reappearance of mucus (thin strands)