

## 24 PREPARATION OF VAGINAL SMEARS IN RAT/MOUSE

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### 24.1 INTRODUCTION

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You have learnt in Unit 8, block 2 of LSE-05 course that gamete production in female vertebrates is cyclic and in most animals takes place during seasons which are most favourable for the survival of their offspring. In mammals, other than primates, the reproductive cycle is known as **estrous cycle**.

Rats and mice have an estrous cycle of 4 to 5 days and the cycle is divided roughly into four stages.

(a) **Estrous:** This is the period of heat during which ovulation occurs. This period lasts for 9-15 hours under the influence of follicle stimulating hormone (FSH) and estrogen. The female is receptive to the male only during this period; therefore, ovulation and fertilisation are well coordinated. The uterus becomes enlarged and the vaginal mucosa proliferates and the vaginal epithelium becomes squamous and cornified. A vaginal smear taken during this period shows **squamous cells** indicative of estrous.

Subsequent events depend on sexual contact with male.

2. **Metaestrous:** In the absence of copulation this stage occurs shortly after ovulation and lasts for 10 to 14 hours. A small corpus luteum is formed and some progesterone is secreted. A vaginal smear taken at this stage shows **leucocytes with some cornified cells**.
3. **Diestrous:** This stage lasts for 60 to 70 hours. The corpora lutea regress during this period and vaginal smear contains **only leucocytes**.
4. **Proestrous:** Lasts for about 12 hours. It precedes the next estrus. Degeneration of old corpora lutea continues but new follicles mature rapidly. The uterus becomes distended again and the vaginal smear contains individual **nucleated epithelial cells** or in sheets.

All these changes in vaginal lining are shown diagrammatically in Fig. 24.1.

**Objectives:** In this experiment you will be able to:

- prepare vaginal smears from rats or mice and
- identify the stages in the estrous cycle in the animals provided to you.

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### 24.2 MATERIALS REQUIRED

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Female rats/mice  
Physiological saline  
Cotton swabs  
Giemsa stain  
Microslides  
Coverslips  
DPX mountant

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### 24.3 PROCEDURE

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Anesthetise the rats/mice with chloroform. As soon as they are immobilised, lay them on

a dissection tray with the ventral side up. Use a cotton swab (preferably use an ear-cleaning cotton bud) moistened with physiological saline. Insert it into the vagina of the animal and roll it gently. The cells adhering to the lining of the vaginal wall will stick to the moist cotton swab.

Remove the cotton swab and smear it on a clean slide. Dry the slide by waving in air for a few minutes. Leave the preparation in a petridish and add a small quantity of Giemsa stain to the smear. Cover and leave for 10 minutes. Wash the slide well in distilled water. Dry it in air. Mount the slide with a coverslip using DPX mountant.

## 24.4 OBSERVATIONS AND RESULTS

Observe under the microscope and identify the stage of the estrous cycle on your slide with the help of the description given in the introduction and the figure given below. Draw the stage you have identified on your slide in the note book.

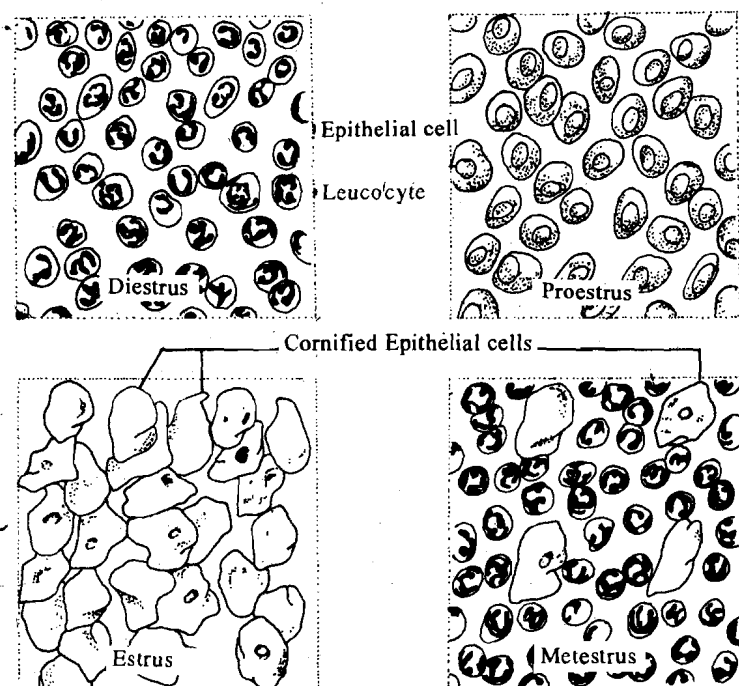


Fig. 24.1 : Different stages in the estrous cycle with their characteristic cells seen in vaginal smears.

Since the animals have been anaesthetised using chloroform, they would recover soon. Therefore, after taking the smear return them to their cages.

You can make several such vaginal smears using the same animals at 2-3 days intervals over the two week period of your stay at the study centre.

1. What is the stage of estrous cycle in which you find your animal?

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2. What are the types of cells observed in the stage you have identified?

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3. Fill in the following table with the data from the other students.

STAGE OF CYCLE	ESTROUS	METAESTROUS	Diestrous	PROESTROUS
NUMBERS OF ANIMALS				

4. Do you find more number of animals in any particular stage of the estrous cycle? If yes, could you give possible reasons for such an observation.

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