Processing and double staining of different stages of Oestrous Cycle in Rat

Introduction:

Oestrous cycle, also called the Estrous cycle, is a hormonally controlled cycle of activity of the reproductive system of non-primate Therian females. This includes a definite cyclic change in hormone, ovary, uterus, vagina, and also in behaviour. In rats, estrous cycles throughout the year; rats and animals such as mice, pigs, and cattle that experience multiple estruses in a year are called poly-estrous animals. Horse, sheep, goats, deer, and cats experience multiple estrous but only in a certain period of a year; such animals are called Seasonally Poly-estrous animals. Animals such as dogs, wolves, foxes, and bears that experience only one estrous per year and are called mono-estrous animals. Typically, the cycle consists of four phases, duration and nature of each phase may vary in different species.

Table 1:Different Phases of Oestrous cycle in Rat

Stages	(Duration in hours)	Ovary	Vaginal Smear
Proestrus	12	Follicular growth	Nucleated epithelial cells appear
Estrous	30	Ovulation	Cornified epithelial cells appear
Metestrus	6	Corpora lutea formation	Leucocytes
Diestrus	50-55	Corpora lutea active; diminishes in absence of pregnancy at the end of this phase.	Nucleated epithelial cells start reappearing alongside leucocytes.

An additional phase called the Anestrus phase is also observed, it represents the interval of sexual inactivity between two periods of estrous in mono-estrous animals. The inactive period during pregnancy, lactation, and illness is also sometimes included under this term.

Requirements:

I. Healthy sexually mature female rat (about 5 weeks to less than 18 months of age).
II. Normal saline
III. Double distilled Water.
IV. Micropipette with 200 µl tip
V. Slide, coverslip
VI. Mayer's Albumin
VII. Double staining set
VIII. Microscope

Procedure:

Preparing the animal

Prior to the experiment healthy female rats of appropriate age were separated from males, to avoid pregnancy, and were housed in separate cages. (Female rats should be acclimatized for a period of two weeks to identify the already pregnant ones). If sexing had already been done before 5 weeks of age, animals were acclimatized for 7 days only. Institutes Animal Ethical committee guideline was followed for all the procedures required in this experiment.

Vaginal cell collection

1. The rare end of the mouse was lifted by grasping the tail. Urine (if any) from the vaginal opening was rinsed using ddH2O (not using the sample collection tip).
2. Vaginal cells were collected using a separate pipette tip by slowly placing and drawing the normal saline from the vaginal opening (without penetrating the orifice; to avoid pseudopregnancy). *The normal saline will be spontaneously aspirated by the animal.
3. The fluid (normal saline with vaginal cells) thus collected was used for the next step.

Vaginal smear Preparation:

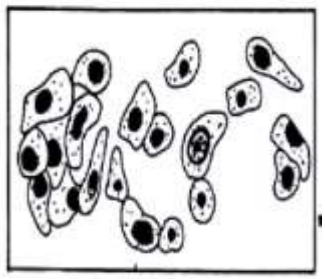
1. Mayer's Albumin was applied on slides.
2. Few drops of fluid were placed at the centre of the albumin coated slides and a thin smear was prepared.
3. The smear was air-dried and fixed with methanol and used for the next step.

Double staining of the vaginal smear.

1. The smear was stained using the standard double staining Protocol
   Haematoxyline → dehydration using alcohol grade up to 90% → Eosin → 90% wash → 90% → 100% X 2 → Xylene X 2 → mounting in DPX.
2. The slide was observed under a microscope.

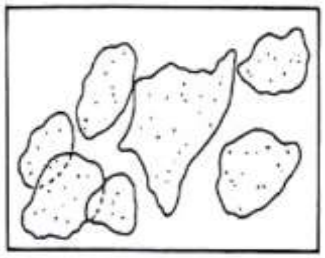
Identifying characteristics:

Proestrus



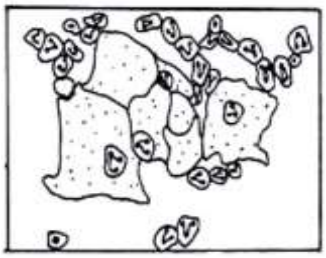
Cornified epithelial cells- Absent or a Few (in case of late proestrus)
Nucleated epithelial cells-Predominant
Leucocytes- Absent

Estrous



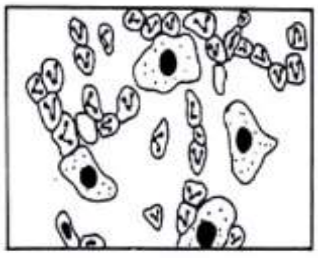
Cornified epithelial cells- Predominant
Nucleated epithelial cells- Absent or rare
Leucocytes- Absent

Metestrus



Cornified epithelial cells- Present
Nucleated epithelial cells- Absent
Leucocytes-Present

Diestrus



Cornified epithelial cells- Few
Nucleated epithelial cells- Few
Leucocytes- Predominant

Observation-

Describe what you have observed with diagram

Inference-

Infer on the basis of the observation.