## EXAMINATION OF VAGINAL SMEARS IN THE RAT

4 Phases: <u>Pro-oestrus</u> <u>Oestrus</u> <u>Metoestrus</u> <u>Dioestrus</u>



At this stage (DIOESTRUS) of the oestrous cycle there is little material to be collected, and it consists *mainly of traces of secretory material with cellular debris*. There are few intact cells to be found. Some parabasal and intermediary cells may be observed. There are *usually few leucocytes*, if any, to be seen.



- The diestrous smear consists mainly of leucocytes (polymorphonuclear) and some epithelial cells (nucleated, much larger than the leucocytes).
- The number of nucleated epithelial cells increases during proestrus and early estrus; leucocytes disappear.
- In full estrus, which lasts but a few hours, the smear consists entirely of large cornified epithelial cells (non-nucleated).
- Leucocytes appear again in metestrus, together with some remaining cornified epithelial cells.

**Transition from Dioestrus to Pro-oestrus:** There is considerably more mucus at this stage, often present as thick strands or discs. The histological picture is more complex and darker. Leucocytes are rarely seen in pro-oestrus. Most of the cells present are parabasal cells, often with an irregular or shrunken appearance, but intermediary cells may also be observed.



**Early Pro-oestrus:** The smear becomes "cleaner", with less mucus. Parabasal cells appear more clearly, as do intermediary cells. Cell debris can still be observed.



**Pro-oestrus:** The smear is much lighter in colour and mucus is rarely seen. The cells are predominately intermediary cells, and parabasal cells are rare. Lecocytes are hardly ever observed.



**The Transition from Pro-oestrus to Oestrus:** The smear is clear and dominated by cells. These consist of intermediary cells, superficial cells and anuclear (keratinised) cells.



**Oestrus:** The smear consists nearly entirely of keratinised superficial cells that lie singly in early oestrus. They form groups as oestrus progresses and by the end of this stage of the cycle they can form large flakes.



**The Transition from Oestrus to Metoestrus:** Although flakes of keratinized cells are still present, this stage is characterized by the presence of leucocytes and (to a lesser extent) intermediary cells.



**Metoestrus:** The picture at this stage of the cycle is dominated by leucocytes, often in large numbers, and intermediary cells.



**Metoestrus:** As this stage progresses, more intermediary cells begin to appear. These are often small and dark. Parabasal cells can also be seen. However, larger intermediary cells and leucocytes are also present.



**The Transition from Metoestrus to Dioestrus:** This stage is characterised by the reduction in cell numbers and the reappearance of mucus. often in thin strands.



The Transition from Metoestrus to Dioestrus: This picture shows a continuation of this process, as the mucus becomes progressively more apparent and cell numbers decline.



Anoestrus: The cellular picture at this stage resembles in many ways the transition from dioestrus to pro-oestrus, but the general picture is darker, the cells rarely appear intact and there is a lot of mucus and cellular debris. It is often at this stage that the rat's oestrous cycle may halt.



- During the anestrus portion of the cycle, the smear consists preponderantly of leucocytes, with an occasional cornified epithelial cell.
- The first appearance of estrus is marked by mucification of the vagina, followed quickly by the complete, or nearly complete, disappearance of leucocytes and their replacement by large numbers of round, nucleated, epithelial cells.
- These cells are about three times as large as the leucocytes and, once seen, cannot be mistaken. At this time and for about 12 hours afterward, the animals will breed, but at no other time.
- Desquamation of the epithelial cells now occurs, and white, cheesy masses of disintegrating squamous cells are found in the smear. Leucocytes now again make their appearance, and the cycle is repeated.

**The Transition from Pro-oestrus to Oestrus:** This picture is characteristic for early oestrous development after a period of anoestrus. The vaginal smear may in some cases show fewer cells than in this picture.



 Reproduction in Mammals: Hormonal Control of Reproduction; By Colin Russell Austin, Roger Valentine Short; Published by Cambridge University Press, 1984; ISBN 0521275946

## **Development of Rat Follicular Cells**

- Duration of follicle recruitment and selection in human and rat ovaries. Primordial follicles undergo initial recruitment to enter the growing pool of primary follicles. Due to its protracted nature, the duration required for this step is unknown.
- In the human ovary, greater than 120 days are required for the primary follicles to reach the secondary follicle stage, whereas 71 days are needed to grow from the secondary to the early antral stage.
- During cyclic recruitment, increases in circulating FSH allow a cohort of antral follicles (2–5 mm in diameter) to escape apoptotic demise.
- Among this cohort, a leading follicle emerges as dominant by secreting high levels of estrogens and inhibins to suppress pituitary FSH release.
- The result is a negative selection of the remaining cohort, leading to its ultimate demise. Concomitantly, increases in local growth factors and vasculature allow a positive selection of the dominant follicle, thus ensuring its final growth and eventual ovulation.
- After cyclic recruitment, it takes only 2 weeks for an antral follicle to become a dominant Graafian follicle.
- In the rat, the duration of follicle development is much shorter than that needed for human follicles.
- The time required between the initial recruitment of a primordial follicle and its growth to the secondary stage is more than 30 days, whereas the time for a secondary follicle to reach the early antral stage is about 28 days.
- Once reaching the early antral stage (0.2–0.4 in diameter), the follicles are subjected to cyclic recruitment, and only 2–3 days are needed for them to grow into preovulatory follicles.

















## Rat Embryo Development (Pre-implantation)

Age (days)	Size(mm)	Stage
1	0.07	zygote
2	0.08x0.06	2-cell
3	0.08x0.05	4-cell
3.5		8-12 cell
3.75	0.08x0.04	morula
4	0.08x0.03	early BL
5	0.12x0.05	BL

http://embryology.med.unsw.edu.au/OtherEmb/Rat.htm