

Reproductive Problems in the Bitch : Finding answers through Vaginal Cytology

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Infertility in the bitch is a difficult problem to investigate, because oestrus is infrequent, details of breeding are often unavailable, and many of the reproductive organs are inaccessible on physical examination. The oestrous cycle of the bitch has been divided into four phases, i.e., pro-oestrus, oestrus, metoestrus/dioestrus and anoestrus. This article is intended to describe the use of vaginal cytology as a tool in staging the oestrous cycle. When we are misled by the bitch's behaviour, or in doubt about the significance of her discharge or wonder whether she is really in oestrus because of a marginal amount of vulvar swelling, cytology can always be a help to define her reproductive status.

Principle

Vaginal epithelium is responsive to changing levels of oestradiol. It changes from a bistratified cuboidal epithelium to a stratified squamous epithelium of many layers during the follicular phase of oestrous cycle. When we examine a stained vaginal smear we are actually observing the cells exfoliated from the vaginal epithelium at that particular time. Thus the changes in exfoliate vaginal cells observed can provide a direct evidence of follicular development and oestrogen secretion during pro-oestrus, a reasonable estimate of the fertile period, and fairly accurate estimates of the end of the fertile period and the timing of subsequent events of a resulting pregnancy. However, it should be stressed that this examination must be supported by a careful history and clinical examination.

Preparation of vaginal smears

For sample collection, the bitch should be placed on a table either in standing position or on lateral recumbency. There are two methods for sample collection.

1. Pipette method: In this method a sterile blunt-ended glass pipette with a rubber bulb or plastic syringe connected by rubber adapter is used. A small column of saline (approximately 1 ml) is drawn up into the pipette. It is then introduced into the

vagina, carefully directing the pipette upwards to the vestibule and at the vestibulo-vaginal junction the pipette is redirected cranially into the anterior vagina (Fig. 1). The bulb is squeezed quickly, gently and partially several times, to make the fluid column wash rapidly back and forth to pick up vaginal cells. The pipette is then withdrawn carefully avoiding pressure on the bulb. Place a small drop of fluid on the slide and prepare a thin smear and air dry.

2. Swab method: The cotton swab should be moistened with saline solution. A speculum should preferably be used to prevent contact between the swab and the lips of vulva and vestibule. Introduce the moistened swab through the sides of the speculum into the vagina and twist a full turn to pick up a sample of cells. Withdraw the swab. Place the swab on the slide and gently roll it across the surface. If a speculum is not available the swab should be introduced through the dorsal commissure so as to avoid the clitoral fossa.

Staining technique

Any blood or general cytology stain such as Wright's, Leishman's, Giemsa, Wright-Giemsa, or New methylene blue may be used. Special stains like papanicolaou and Shorr are easy for interpretation but are quite cumbersome. The smears are examined microscopically under high power or oil immersion objective.

Classification of vaginal cells

The exfoliated vaginal epithelial cells are classified into 9 cell types according to Christie et al. (1972) under the broad categories: parabasal, intermediate, superficial and anuclear.

Parabasal: These cells are round or oval and are the smallest epithelial cells commonly observed in smears. They are the least mature of cells exfoliated. The diameter of their nucleus is usually greater than 45 per cent of the cells diameter and it can be as large as 90 per cent of the cells diameter. They are numbered as cell number 9 and 8 according to their size; cell type 8 being a

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little larger.

Intermediate: These include a wide range of sizes and types because they represent all stages of maturation between parabasal and fully mature superficial cells. The less mature intermediate cells are small and polygonal with a relatively large nucleus (Cell No.7). They become more angular, enlarge and flatten as they mature. In some

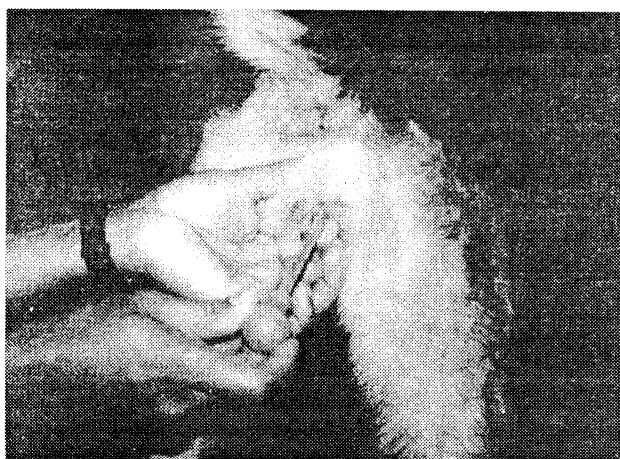


Fig - 1, Collection of exfoliate vaginal cells using a glass pipette

large intermediate cells, the nucleus may contain small opaque foci eventhough they have normal architecture (cell No.6) The relative size of the nucleus decreases as the cells enlarge (Cell No.5)

Superficial cells: These are polygonal cells with no normal architecture for the nucleus. They are of three types based on nuclear appearance: those with pyknotic nucleus (Cell No.4), those with nuclear membrane only (Cell No.3) and those having small opaque foci in the place of nucleus (Cell No.2).

Anuclear/anuclear superficial : The

nucleus undergoes karyolysis as the cells mature and finally they become absent in the anuclear superficial. These are large, polygonal, dead, irregular vaginal cells with no nucleus (Cell No.1). For routine interpretation anuclear cells are also counted along with superficiaals.

Patterns during the oestrous cycle

Vaginal cytology can be used to monitor the oestrous cycle, especially the follicular phase of pro-oestrus and oestrus (Table 1). During pro-oestrus, the parabasal, intermediate and some superficial cells are exfoliated. Red blood cells, WBCs, and bacteria are present. During pro-oestrus the percentage of superficial cells increases until it is nearly 100 per cent by the beginning of oestrus. The percentage plateaus for a period of 10 to 14 days. Background debris are usually absent during oestrus (Fig.2). An abrupt clearing of the background usually indicates the occurrence of LH surge. Red blood cells may or may not be present. The number of bacteria are fairly low during oestrus as the oestradiol levels are high and the vaginal epithelium is cornified.

The transition from late oestrus to early metoestrus is dramatic. The parabasal cells and intermediate cells reappear in the vaginal smear. The first day of the reappearance of these cells and the decline of superficial cells is by definition referred to as the onset of diestrus or metoestrus. In majority of cycles there is a concurrent reappearance of neutrophils in moderate to abundant numbers at the same time as the metoestrous shift in epithelial cells. During anoestrus, the epithelial cells are few to moderate in number. The cells are mostly parabasal and small intermediates. Neutrophils are usually present, but not numerous.

Clinical usefulness of vaginal cytology

1. Breeding management: Determination of different stages of the oestrous cycle can help to determine optimal breeding dates in order to maximize conception and litter size. The vaginal smear will be of the superficial type (90-100%)

Table 1. Vaginal cytologic findings during oestrous cycle of a normal bitch

	Pro-oestrus	Oestrus	Metoestrus
Parabasal	+	-	+
Intermediate	+	few/-	+
Superficial	rare	≥/-	-/+
RBC	++	+/-	-/+
WBC	+	-	++/+

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during the entire fertile period in normal bitches. Maximal conception rates and litter size are obtained when normal bitches are bred on the first day of oestrus and again at 3-4 days interval till the bitch ceases to accept the male. Breeding on alternate days is also acceptable.

Various indices like superficial cell index, eosinophilic index, karyopyknotic index, cornification index and anuclear cell index have been suggested for finding the optimum time for breeding. The anuclear cell index offers advantages over other methods for better breeding management. Here, the percentage of cell types 1, 2, and 3 according to Christie et al. (1972) is calculated as "anuclear cell index". Here the animal should be examined on a daily or alternate day basis. It is recommended that bitches be bred two days after the first anuclear peak and a second mating may be given 3 or 4 days after the first mating.

2. Whelping management: Vaginal cytology is a superb tool in predicting the approximate date of whelping. Normal gestation length when calculated based on breeding dates varies from 57 to 72 days after the first mating date. This is because ovulation occurs at variable and unpredictable times during behavioural or cytologic oestrus, and the canine sperm maintains its ability to fertilize for at least 4 to 6 days in the oestral tract. When gestation length is calculated from the first cytologic day of dioestrus, whelping occurs

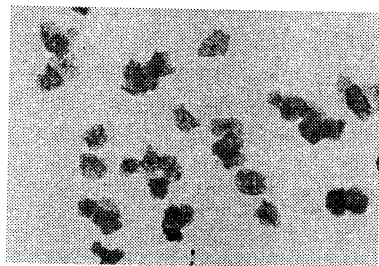


Fig - 2, Vaginal smear from a bitch in oestrus revealing superficial epithelial cells.

near day 57 of dioestrus in almost all bitches. This knowledge is quite helpful in managing problem pregnancies.

3. Mismatching: The owner may not actually observe an unwanted mating. Vaginal cytology would help to determine if a bitch has recently been bred, since sperm heads (not intact sperm) are detected in vaginal smears from 68% of bitches bred 24 hours earlier, and from 50% of bitches bred 48 hours earlier. However, lack of these findings does not eliminate the possibility that a breeding took place. Vaginal cytology is

indicated before administration of oestrogens as they are contraindicated if the bitch has passed on to dioestrus stage. This is because pyometra occurs in 25% of bitches given oestradiol during dioestrus. Also, if the mating took place while the bitch was in pro-oestrus stage there is no need to worry about the occurrence of a fertile mating as sperm survival in the vagina is poor in this stage.

4. Vaginitis: Vaginitis is diagnosed by a combination of clinical signs and cytology. The vaginal smear from a bitch with vaginitis will be marked by the presence of degenerated epithelial cells and degenerated (reactive) neutrophils that have ingested bacteria or cellular debris. Large colonies of bacteria may also be present in greater concentration than the normal vaginal smear. The number of neutrophils are also greatly increased.

5. Unusual cycles: Some bitches having normal reproductive cycles do not stand to be mated. Many of them fail to stand for a male because they are not exposed to the stud during standing heat. This might be because of an unusually short or long pro-oestrus and the owner fails to recognize the correct time. Pro-oestrus can be as short as 3 days or as long as 3 weeks. In other cases, where the bitch refuses natural breeding artificial insemination can be carried out based on vaginal cytology.

6. Follicular cysts: A bitch with a follicular cyst appears to have a prolonged pro-oestrus and/or prolonged oestrus. This persistent evidence of pro-oestrus/oestrus can be diagnosed using vaginal cytology.

7. Pyometra and acute metritis: Animals with open-cervix pyometra or metritis may have large numbers of degenerated neutrophils and, occasionally, vacuolated endometrial cells. However, these two conditions are serious problems that usually cause systemic illness and therefore, vaginal cytology is not the most reliable diagnostic aid for these disorders.

8. Vaginal tumours: some tumours may be recognized by vaginal cytology. These include transmissible venereal tumour and transitional cell carcinoma of the bladder which may extend into vagina via the urethra.