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## Exfoliative Vaginal Cytology (EVC) as a tool for determining breeding time in the bitch a case study

**Rouf Rashid Dar, Mohd Yaqoob Bhat, Pooja Dogra, Amir Amin Sheikh, Rakshanda Bhagat, Ajaz Ali and Wani Ilyas**

**Abstract**

A three year old German shepherd bitch was presented to Teaching Veterinary clinical complex, International Institute of Veterinary Education and Research (IIVER), Rohtak Haryana, with history of frank reddish brown, non fetid, vaginal discharge from last 12 days with acceptance to male and cessation of discharge on the day of arrival to clinics. Her owner, wanted to know the window of fertile mating. Exfoliative vaginal cytology (EVC) technique was done to obtain serial vaginal smears, which were stained with Giemsa and put to microscopical examination for presence or absence of cornfield (Squamous and Anucleate epithelial cells)/non cornified (Parabasal and Intermediate epithelial cells) population of vaginal cells to know the fertile window of possible mating. Owner was advised to go for mating of the receptive bitch on the prescribed window based on the presence of typical cornified superficial and anucleate cells in vaginal smear.

**Keywords:** Cytology, discharge, exfoliative, mating, vaginal smears

**1. Introduction**

Exfoliative vaginal cytology as an essential part of the gynaecological examination [1,2,3,4,5,6,7] is a simple, non-invasive method for the determination of the phases of the oestrous cycle (anoestrus, proestrus/oestrus, metoestrus) [4] and is additionally applied in cases of silent heat [8,9] or suspected ovarian cysts, ovarian remnant syndrome, postpartal disturbances in the endometrial involution or Sticker sarcoma. The exfoliated cells reflect the hormonal, in particular the estrogenic state, of the bitch [8]. Due to the estrogenic influence, an increase in cell layers, keratinisation and exfoliation is observed in the follicular phase during proestrus, such that the 3-4 layered epithelium in anoestrus becomes 20-layered during oestrus. The cells change characteristically in size and nuclear morphology. In anoestrus, predominantly parabasal cells with a large nucleus and homogenous cytoplasm are found. During early proestrus, single parabasal cells are identified among erythrocytes and intermediate cells. As this phase progresses, the percentage of large intermediate cells and nucleated superficial cells increases. The oestrus is characterised by a high cell number, initially superficial cells with pyknotic nuclei, later anucleated squamous cells that are located in cell nests. The switch to metoestrus is associated with a large number of neutrophil granulocytes and a sudden change of cytology within 24-48 hours. Vaginal cytology can be performed in any practice due to its simplicity and the limited equipment necessary (speculum, cotton wool wad, slide, staining and microscope). Because the results are rapidly available, it is a useful addition to gynaecological examination to differentiate the stage of the cycle (anoestrus, proestrus/oestrus, and metoestrus) and to diagnose infectious, inflammatory and tumorous conditions in the bitch. Performing vaginal cytology offers a rapid, inexpensive, and reliable in-clinic method to evaluate stages of the estrus cycle in the bitch. Veterinary discomfort with obtaining and interpreting vaginal cytology is common; submission to a commercial laboratory might result in diagnostic delays and increased client costs.

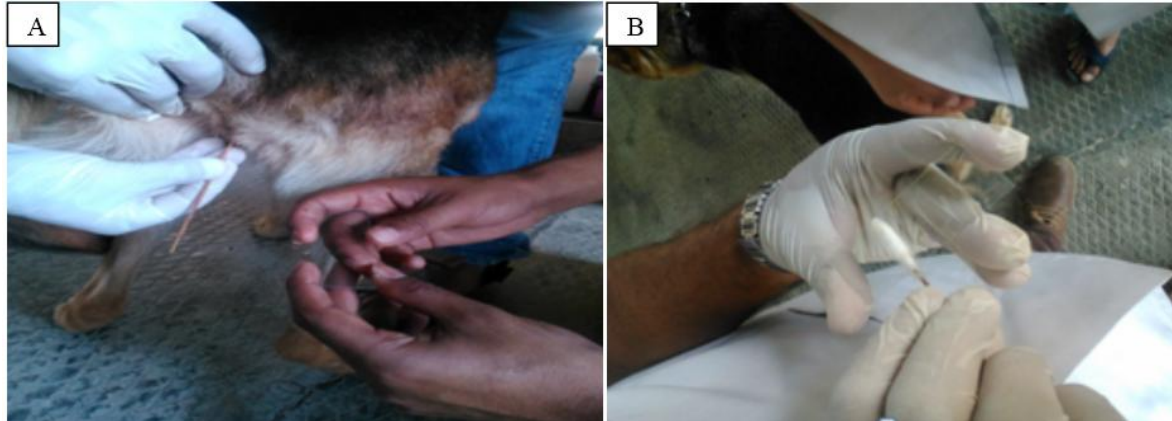
**2. Material and methods**

**2.1 Case history**

A three year old German shepherd bitch had a history of vulvar edema, frank reddish brown, non fetid, vaginal discharge from last 12 days with acceptance to male and cessation of discharge on the day of arrival to clinics.

Her owner, wanted to know the window of fertile mating. The sample was collected from the cranial vagina because cells from the clitoral fossa, vestibule, urethral papilla, or vestibulo vaginal junction are not as indicative of the stage of the cycle and provide confusing results. A cotton-tipped applicator<sup>[8, 9, 10]</sup> (Fig. 1) (moistened with water if needed) was passed into the vulva in a dorsal direction and advanced horizontally above the clitoral fossa and urethral papilla into the vagina, which is at the level of the cranial thigh. After swabbing (by

gently rubbing or rolling) the vaginal wall, the applicator was removed and rolled (not smeared) onto a glass slide<sup>[11, 12, 13]</sup> (Fig. 1). Giemsa staining was performed after methanol fixing and air-drying the slide. Slide was scanned at low power first (40×) and high power as necessary (100×) to aid in particular cell identification. This procedure was repeated on alternate days for three days till cytological evidence of diestrus phase characterized by the presence of parabasal cells and neutrophils in vaginal smears.



**Fig 1:** Diagram showing vaginal swab being introduced in to the vagina (A) of a bitch and its rolling on a glass slide (B) for observing different stages of vaginal epithelial cells in EVC technique.

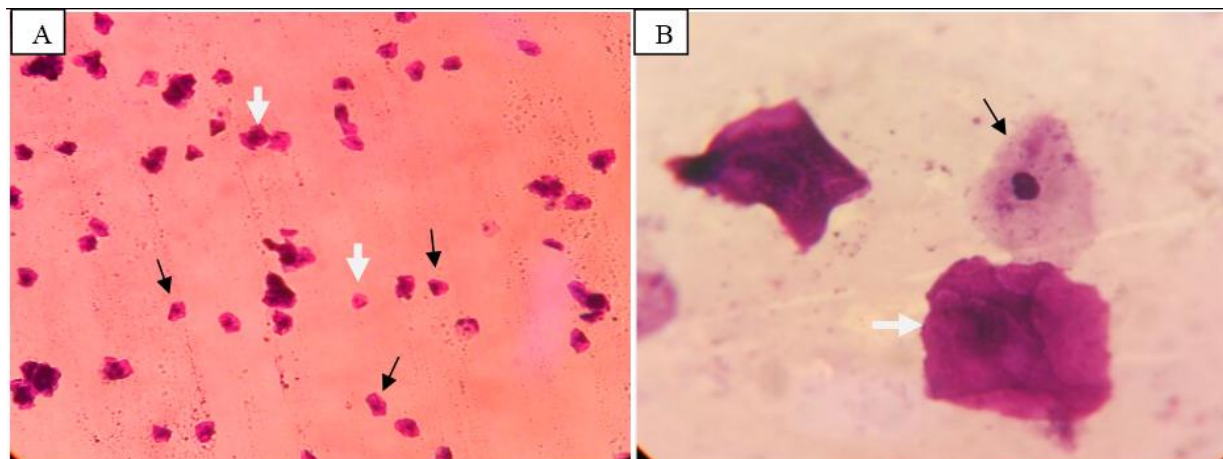
**3. Results**

On serial microscopic examination of the vaginal smears for three days alternatively, it was observed that the 80 per cent of the vaginal epithelial cells were cornified population<sup>[12, 14, 15]</sup> of squamous and anucleate cells (Fig. 2 A, B) with more than 50 per cent cornified anucleate ones showing folded borders and pyknotic nuclei (Fig. 2 A). All the above findings along with the history of profuse serrosanguinous vaginal discharge for around 12 days are suggestive of bitch being in late proestrous to early estrous stage of the cycle. Thus the owner was advised to go for breeding of the bitch on three alternate days starting with the day vaginal smears showed

cornified population of vaginal epithelial cells till cytological evidence of diestrus phase<sup>[14, 15]</sup> in vaginal smears.

**4. Discussion**

Stages of the canine estrous cycle can be defined by sexual behavior, physical signs (vulvar swelling, vaginal bleeding) or by vaginal cytology. The period of receptivity to a male varies considerably among bitches; some bitches are receptive well before and after the period of potential fertility. Similarly, signs such as "proestrus bleeding" are often unreliable indicators; some bitches bleed very little and other show bleeding through estrus and into diestrus.



**Fig 2:** Vaginal smear showing vaginal population of cornified squamous (black arrow) and anuclear epithelial cells (white arrow), under Giemsa 40x (A) and 100x (B).

Since cytologic changes reflect the underlying endocrine events of the cycle, they are almost always a better predictor of the "fertile time" and gestation length than are behavioral or physical signs. Cytologic changes through the canine estrous cycle reflect changes in blood concentrations of estrogen. Estrogen levels rise prior to and during proestrus and fall in conjunction with the preovulatory surge of

luteinizing hormone. Rising levels of estrogen induce the "cornification" that is characteristic of smears examined during estrus<sup>[8]</sup>. Ovulation occurs two days after the LH surge. Serum concentrations of estrogen rise during proestrus, leading to capillary breakage and leakage of red blood cells through uterine epithelium, as well as proliferation of the vaginal epithelium. Examination of vaginal smears from early

to late proestrus will reveal a gradual shift from intermediate and parabasal cells to superficial cells. Typically, red blood cells are present in large numbers and neutrophils are commonly observed. Large numbers of bacteria are also often present in this phase. The defining characteristic of cytologic estrus is the predominance of superficial cells. Most, but not all, bitches will undergo full cornification, and the smear will reveal a monotonous pattern composed almost exclusively of anucleate superficial cells. The onset of diestrus is marked by a precipitous decline in the number of superficial cells and reappearance of intermediate and parabasal cells<sup>[14, 15]</sup>. Most commonly, the cellular profile changes within a single day from essentially 100% superficial cells to less than 20% superficial cells<sup>[14, 15]</sup>. However, it is best to confirm the onset of diestrus by examining a smear prepared on diestrus day 2. The significance of identifying the onset of diestrus is that it is a considerably more accurate predictor of the time of ovulation, and hence gestation length, than sexual behavior. Dogs ovulate 5-7 days prior to the onset of diestrus (7-9 days after the preovulatory LH surge), and hence, gestation length is usually  $57 \pm 1$  day from the onset of diestrus day 1<sup>[12, 16]</sup>. The period of behavioral estrus is variable, and often extends up to several days before and/or after cytologic estrus. Gestation lengths calculated from the onset or cessation of receptivity is correspondingly inaccurate. The onset of diestrus also correlates well with loss of fertility, and breedings after the diestrus shift are rarely fertile.

## 5. Conclusion

In the present case, serial vaginal smears were collected and microscopically examined from the receptive bitch for three days alternatively and was concluded that 80% of the vaginal epithelial cells were squamous and anucleate cornified cells containing 50% cornified anucleate ones with pyknotic nuclei and folded borders. All the above findings along with the history of profuse serrosanguinous vaginal discharge for around 12 days are indicative of bitch being in late proestrus to early estrous stage of the cycle. Thus the owner was advised to go for breeding of the bitch on three alternate days starting with the day vaginal smears showed cornified population of vaginal epithelial cells till cytological evidence of diestrus phase.

## 6. Acknowledgement

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