

## Exfoliative Vaginal Cytology for Optimum Breeding time Determination in French Mastiff Bitch

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### Abstract

The knowledge of reproductive physiology of bitch in estrus cycle is important for animal management and to determine the reproductive potential of animals. For that purpose exfoliative vaginal cytology in bitches can be used by practitioners to know the proper stage of reproductive cycle, disease condition of genital tract and also useful in determination of optimum mating timing in bitches, present case report suggests the optimum timing of service for excellent conception rate in bitches.

**Keywords:** Bitch; Estrus; Ovulation; Optimum breeding time; Exfoliative vaginal cytology; Conception rate; Swab method.

### INTRODUCTION

Vaginal exfoliative cytology is the inexpensive, rapid, and reliable, method to evaluate stages of estrus cycle in bitch (Romagnoli, 2017). It is the most popular diagnostic method as a part of

gynecological examination in the bitch (Groppetti *et al.*, 2012; Wehrend *et al.*, 2013). It is based on cyclic cellular changes occurring in vaginal epithelium as a result of reproductive hormone that is estrogen (Write and Parry, 1989).

There are four phases of canine estrus cycle proestrus, estrus, diestrus and metestrus/ anestrus. Here proestrus and estrus stage are called as heat period. For timing of mating and artificial insemination it is important to find the exact date of ovulation during estrus for achieving high fertility rate (Romagnoli, 2017). The diestrus is characterized by slight mucoid discharge which often contains a large number of neutrophils in early stage here the bitch will not allow mounting or breeding of male dogs anymore (Kustritz, 2012)

Symptoms like reddish vaginal discharge, edema of vulva, acceptance of male for mating are the signs of proestrus and estrus (Bunket *et al.*, 2016). The discharge contains large numbers of erythrocytes due to diapedesis through the uterine capillaries due to estrogen effect (Sharma

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and Sharma, 2016). Some of the signs like colour, intensity of edema and moisture of vaginal mucus membrane can be visualised by vaginoscope (Bunk *et al.*, 2016) (Wehrend *et al.*, 2010). Some of the abnormal estrus cycle pattern like early or late ovulation, split/silent heat can also be diagnosed (Goodman, 2001). The cells from vaginal cytology are parabasal, intermediate, superficial, and anuclear. In this parabasal and intermediate are non cornified and superficial and anuclear are cornified (Groppetti *et al.*, 2012; Wehrund *et al.*, 2013)

#### *Different types of cells found in bitch estrus cycle*

**Parabasal cells:** small, round, slightly oval, large vesiculated nucleus and small cytoplasm.

**Intermediate cells:** slightly larger than parabasal cells to twice that size. Smooth, oval to rounded irregular borders, nucleus smaller than in parabasal cells. More cytoplasm than parabasal cells.

**Superficial cells:** Are dead cells, are the largest in vaginal cytology with a sharp, flat, angular cytoplasmic borders and a small pyknotic, fading nuclei or without nuclei.

**Anuclear:** irregular vaginal cells, no nucleus, smaller than superficial. These are the cells that have also been called "fully cornified."

**Metestrus cells:** Large intermediate vaginal cells that appear to have one or more neutrophils in their cytoplasm. Are typical of bitch diestrus.

**Foam cells:** parabasal and intermediate cells with obvious cytoplasmic vacuoles. May be associated with diestrus and anestrus.

**Neutrophils:** Inflammatory cells that can be normal or abnormal in vaginal cytology in function of the estrus period.

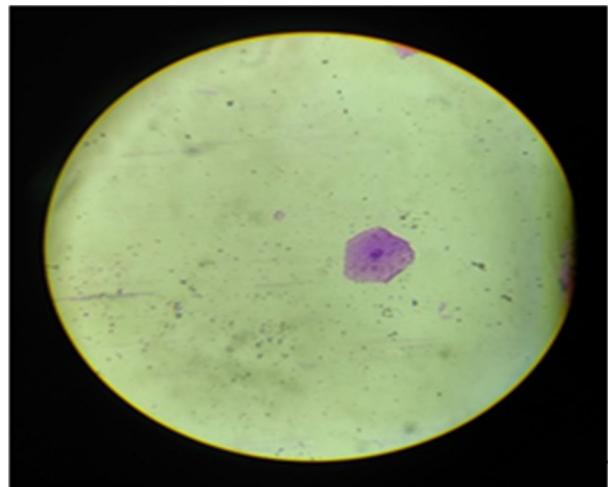
**Red blood cells:** Blood can be normal in the bitch.

#### *Case history and observation*

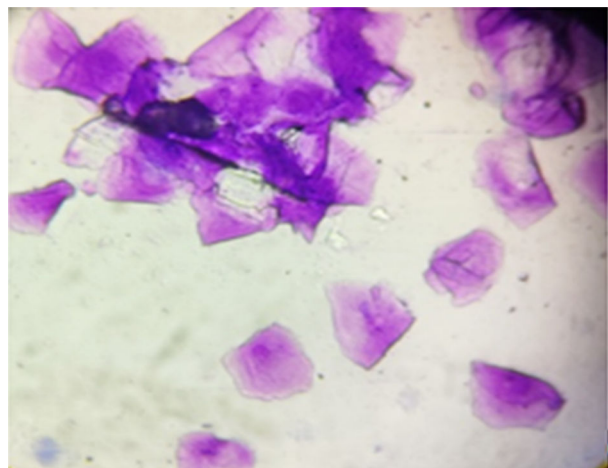
Three year old female French mastiff present at veterinary complex parbhani with the complaint of unable to conceive from last three consecutive estrus cycle and with a history of bloody discharge from last four to five days. On clinical examination we noticed that there is slight vulval swelling as it's a stage of estrus cycle. So owner wants to know the proper timing of mating for the successful conception.

## MATERIAL AND METHODS

Cotton swab, Slide, Field stain A and B is the material used for exfoliative vaginal cytology. The method used to obtain good vaginal cytology was the application of cotton swab in the cranial vagina of a bitch. The lip of the vulva were gently separated with the fingers and a cotton swab was passed into dorsal aspect of the vulva. Clitoris fossa was avoided and advance in a cranio-dorsal direction, towards the vertebral column at this point the cotton swab was rotated and withdraw it. The color and type of secretions were observed and rotated the cotton tip on slide. Two or three slides should be fixed every time with the name and date. Lot of stains can be used for vaginal cytology in present case field stain A and B were preferred. After heat drying the slide were set on light microscope on 40X.



**Fig. 1:** Vaginal smear of bitch in late proestrus showing cornified superficial cell (original, MLX-TR Plus LED 40X)



**Fig. 2:** Vaginal smear of bitch in estrus showing cornified nuclear superficial cell (original, MLX-TR Plus LED 40 X)



Fig. 3: Vaginal cytology in French mastiff bitch

## DISCUSSION

The French mastiff bitch, which was used for the study, was found to have a normal vaginal cytology exhibiting normal behavior response to the male dog during estrus phase. In this study, the onset of the proestrus phase in French mastiff bitch was marked by the appearance of serosanguinous secretions on the vulva for a duration of 8 days. The beginning of estrus a normal bitch is days after the first appearance of vaginal discharge (Todorov *et al.*, 2020). From the first appearance of serosanguineous discharge on the vulva, indicating the start of the proestrus phase. Estrous is the phase when the ovulation occurs and the bitch is ready to accept the male dog and mate with him (Orlandi *et al.*, 2021). The phases of proestrus and estrus can be determined by sexual behavior, physical signs (vulvar swelling, vaginal bleeding), or by vaginal cytology (Dar *et al.*, 2017). During estrus, bitches showed a higher percentage of superficial cells (Orlandi *et al.*, 2021). Estrous cycle generally refers to the phase characterized by clinical manifestation, which includes vulvar edema, and sanguineous vaginal discharge, male attractiveness, receptivity to mating, and a cornified vaginal smear.

This timing discrepancy occurs because the individual response to the hormone estrogen differs. Changes in cytology during the dog's estrus cycle correspond to changes in estrogen concentration in the blood (Dar *et al.*, 2017).

As we have discussed earlier the usefulness of vaginal cytology in breeding management in bitches. There may be variation in breeding cycle of bitch, so many breeders and researches may mislead

by this variation. Several factors may impede the dependency of vaginal cytology that includes individual cellular characteristics of the bitch such as variable percentage of Anuclear cells at the time of ovulation (Moxon *et al.*, 2012). Or influx of other cells like neutrophils and erythrocytes (Moxon *et al.*, 2010; Wehrend *et al.*, 2010). Along with the optimal breeding time, anovulation, split heat, false estrus, and source of vaginal bleeding can also be determined. Infectious conditions of reproductive tract and tumors such as lymphosarcoma and transmissible venereal tumor (TVT) can be differentiated from condition like vaginal hyperplasia. The predominance of superficial cells is the defining feature of the cytologic estrus phase (Dar *et al.*, 2017).

The French mastiff bitch allowed males for copulation on the 9th days. It is characterized by the peak of fully cornified cells on vaginal smear examination. On the 9th day of vaginal discharge, fully cornified (anucleated) cells dominated as much as 70%, and the percentage of superficial cells (nucleated) was 30%. Not all female dogs are entirely cornified, and vaginal smear examination consists of nucleated superficial cells, which is consistent with the results of vaginal smear examination of the French mastiff bitch. Thus, on two to three alternate days the bitch should to go for breeding (Kustritz 2012).

There are different methods of doing staining and smears, but there is no standardized evaluation methods may lead to variability in the interpretation (Arlt, 2018; Bergeron *et al.*, 2014).

## CONCLUSION

Due to the accessible equipment and rapid results in canine reproduction the use of the vaginal cytology is simple, cheap and easy to perform. Due to vaginal cytology we can determine the optimal timing of mating. Exfoliative vaginal cytology is valuable in diagnosing various stages of estrus cycle in bitches, various diseases of genital tract and to know the proper mating time of bitches to increase the fertile conception rate in them. It also depicts the hormonal effect like estrogen, progesterone on reproduction and estrus cycle in bitch. Due some kinds of variations in results the evaluator needs to follow standardized procedures to obtain objective and effective results.

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