International Journal of Advanced Research in Engineering Technology & Science
ISSN: 2349-2819
Email: editor@ijarets.org Volume-5, Issue-9 September- 2018 www.ijarets.org

VAGINAL CYTOLOGY FOR DETECTION OF OESTRUS CYCLE STAGES IN BITCH.

Ratnamala Jawale
Assistant Professor, Departent of Zoology,
Veer Wajekas ASC College, Phunde, Uran, Raigad-400702

ABSTRACT

Vaginal samples collection for cytology in bitch is easy quicker and have many applications and importance in the context of gynaecologucal disorders and staging of estrus cycle phases in the bitch. The objective of this article is to describe the use of exfoliative vaginal cytology as a diagnostic tool in staging the cycle of bitch. Total 32 samples from 8 bitches were collected by cotton swab. The mears were stained by Leishman staining and observed under light microscope at 40X. Under microscope Parabasal, Intermediate, keratinised cells were observed in different stage of estrus cycle. The result showed the proportion of vaginal cell was significantly different in each phase of estrus cycle. The proportion of keratinised cell was dominated in estrus phase. In conclusion, Vaginal cytology is a simple, quick and valuable technique for determining the stage of the reproductive cycle by analyzing specific percentages of cell types.
Key words: Bitches, Exfoliative vaginal cytology, Reproductive stages/status.

INTRODUCTION

Knowing the reproductive physiology of the estrous cycle is important for the reproductive capacity of animals. The canine estrous cycle is divided into 4 stages proestrus, estrus, diestrus and anestrous. Proestrus and estrus are commonly called “heat”. Ovulation occurs during estrus and for getting optimum pups it is essential to find an appropriate timing of mating. During the reproductive cycle of bitch, a bloody vaginal discharge with swollen vulva is noticed which will be helpful to determine other stages of the estrus cycle, i.e. proestrus or diestrus (Goodman, 2001). Proestrus can last from 3 days to 3 weeks, with 9 days being the average.

During estrus there were changes in hormone levels in the female. These changes in hormone particularly estrogen from the ovarian follicle, have its effect on vaginal epithelium (Wright & Parry, 1989). Pregnancy is characterized by few intermediate cells, a small number of RBCs, and a moderate to large number of polymorphonuclear cells (Sharma and Sharma 2016) . Thus the changes in the cells of vaginal epithelium gives us an idea of various stages of reproductive cycle. The smear collected over a period of 5-8 days shows changes from parabasal cells, intermediate cells, superficial cells, and anuclear cells. Parabasal and intermediate cells are the non-cornified cells where as superficial and anuclear cells are cornified cells. Therefore exfoliate vaginal cytology is a simple method to detect estrous cycle stages with respect to the optimal breeding time (Turmalay *et al.,* 2011, Groppetti *et al*., 2012; Wehrend *et al*., 2013). This will be beneficial to the owner of dog or breeder for management of breeding of their dogs. Therefore, the study was planned to characterised the various cell types during estrus cycle in bitch.

MATERIALS METHODS

Total 32 vaginal smears from 8 bitches as clinical cases with history of pro-oestrus bleeding presented in the private veterinary clinics located in Navi Mumbai and Uran city were procured during period 2015 to 2016. Vaginal cells were be collected using a sterile cotton-tipped swab stick without a speculum (Johnston *et al.,* 2001). The moist cotton swab is gently inserted into the dorsal commissure of the vulva, advanced caudodorsally, until it passes over the ischial arch. The swab then rotated 2-3 times

in each direction with due care was taken to ensure good contact of swab with the vaginal mucosa. and then withdrawn (Aydin *et al.,* 2011). The collected cells are then lightly rolled onto a glass microscope slide (Feldman & Nelson, 1996; Johnston *et al.,* 2001and Kustritz, 2006).

The vaginal smears were prepared and stained using Leishman"s stain procured from HiMedia Laboratories Private Limited. The Leishman's Staining Technique was performed as described by Christiansen (1984). Briefly, an air-dried smears were fixed in undiluted stain solution for 2 min. The undiluted stain was then added to slide, twice its volume of distilled water was added and mixed gently. The diluted stain was allowed to act for 15 minutes. The slide was washed, dried, and observed under the microscope (40X). A total 100 cells were calculated and were classified by examining the morphology of epithelial cells as parabasal cells, intermediate cells and superficial cells or cornified cells during all the stages of estrus cycle. The data was tabulated in percentages and the Mean ± SE was for all cells were calculated by Microsoft excel.

RESULT AND DISCUSSION

In present study 32, vaginal smears examined from 08 bitches under a microscope (40X) from the start of proestrus bleeding to various stages of their reproductive cycle. It was observed that all smears prepared showed sufficient coloured cells that was easily identifiable as exfoliated epithelial cells and were classified as Parabasal, Intermediate, superficial , Cornified and RBCs Fig. 1 to 2. A total number of 100 cells were counted from each smear and Mean ± SE of cell type was tabulated in table 1. In the present study, Parabasal cells (Fig. 1) are small, round or oval with a large nucleus and with cytoplasmic vacuoles. Parabasal cells may also contain neutrophil granulocytes in the cytoplasm (Wright & Parry, 1989). Similarly larger cells than the parbasal cell having irregular, folded, and angular cytoplasm with few round and polygonal shapes call as intermediate cells (Fig. 1) were seen. Whereas, larger cells (Anuclear cells or Squamous cells or Superficial cells) were found with a pyknotic or faded nuclei with abundant, angular, and folded cytoplasm (Johnston *et al*., 2001) (Fig 2).

The stage of the estrous cycle is commonly identified by the proportion of different vaginal cell types. From table 1, it was found that, as pro-oestrus progresses the parabasal cell changed to intermediate and then in to superficial cells, then superficial cells nucleus gradually lost and became Cornified or superficial cells in estrus.

In this study, the onset of the proestrus phase in bitches was marked by the appearance of serosanguinous secretions on the vulva for a duration of 6-8 days. Vaginal smears prepared during proestrus showed a high intermediate (52.25±1.38) and superficial (43.88±1.59) cells and a small proportion of keratinized cells (2.00±0.40) and red blood cells (Table 1). The similar findings were recorded by Gulavane, (2001) and Root Kustritz, (2006). This pattern occurs because the vaginal mucosa thickens substantially during this phase, creating a barrier that prevents neutrophil migration. In contrast, red blood cells readily enter the vaginal lumen through estrogenic effects on vascular permeability (Reddy *et al*., 2011). This differs from Groppetti *et al*. (2012), who found neutrophils in most smears due to their potential ability to move from uterine capillaries. The authors attributed this presence to neutrophils' capacity to migrate from uterine capillaries into the vaginal canal. Elevated estrogen levels during proestrus enhance vascular permeability in reproductive tissues, facilitating the extravasation of both neutrophils and red blood cells into the vaginal space.

As the proestrus period progresses, vaginal smear examinations show a shift from intermediate and parabasal cells to superficial cells (Gupta *et al*. 2012). In present study, initially, small and large intermediate cells, along with parabasal and superficial cells, are present. By the 7-8th day in most bof bitches, parabasal cells disappear, and superficial and keratinsed cells become predominant. The results of present study differs from the study conducted by Gupta *et al*. (2012), which showed that the proportion of superficial cells was higher during proestrus.

The appearance of maximum number of superficial cells and keratinised (anuclrar) cells is the defining feature of the cytologic estrus phase. Some authors claim that a typical vaginal smear in estrus has 100%; superficial cells and >80 % cells with pyknotic or absent nuclei (Bergeron *et al.,* 2014). Another definition of estrus is the presence of more than 90% superficial keratinized epithelial cells (Bouchard *et al.,* 1991). Most of the bitches between the 9-11 days of vaginal discharge, showed fully cornified (anucleated) cells dominated as much as (65.00±3.16), and the percentage of superficial cells (nucleated) was (29.38±3.79) indicating they are in estrus phae. This is due to estrogen-induced proliferation of uterine and vaginal epithelium (Gupta *et al*., 2012 and Siregar *et al.* 2016). Some bitches in estrus phase showed scanty erythrocytes, absence of neutrophils, and very low numbers of intermediate cells, consistent with earlier findings (Mshelia *et al*., 2001). However, Kustritz (2012) found that not all female dogs show full cornification, with vaginal smears showing nucleated superficial cells, which is consistent with the results of vaginal smear examination of 3 bitches in the present study. Thus, on two to three alternate days the bitch should to go for cytological examination to decide breeding time.

Diestrus occurs in all bitches, irrespective of pregnant or non-pregnant status. This period is of luteal phase of cycle with scanty mucoid vulvar discharge. The onset of diestrus occurs when the number of superficial and squamous cells has decreased by at least 20% (Bouchard *et al.,* 1991) and when a higher number of neutrophil granulocytes are present (Aydin *et al.,* 2011). As per table 1, the females in diestrus showed that the proportion of keratinized cells were reduced drastically while few intermediate (57.25±1.43) and parabasal (29.25±0.84) cells increased. Similar findings were reported by Gulavane, (2001) and Moxon *et al*., (2012). This is due to high progesterone secretion by corpus luteum in the diestrus phase (Zohara *et al.* 2014). In few cases neutrophils were found during this period, this infiltration of neutrophils might be due to a high level of blood progesterone (Gupta *et al.* 2012).

Anestrus is a period of reproductive quiescence and vaginal cytology revealed only a scant number of single cells or small groups of cells i.e., non-cornified (Groppetti *et al.*2015). The nucleated basal (69.50±2.11) and intermediate cells (28.38±1.90) of the stratified squamous epithelium, as well as a few neutrophils, make up the smear in anestrus (Sharma and Sharma 2016).

CONCLUSION

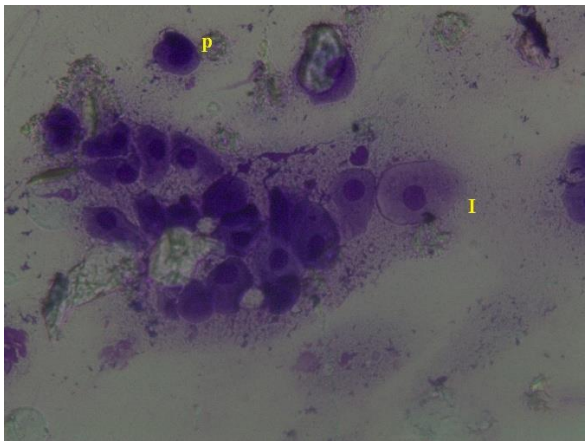
Based on above findings, it is concluded that, vaginal cytology is a simple, quick, affordable and accurate way to assess estrus cycle in dogs. In the present study, based on the presence of anuclear and superficial cells it was indicated that the bitches were in estrus.

Acknowledgement- The authors would like to acknowledge Department of Animal Reproduction, Gynaecology & obstetrics, Mumbai Veterinary College, Parel, Mumbai for providing the help and the facility.

REFERENCES

1. Aydın I, Sur E, Ozaydın T and Dinc DA. (2011). Determination of the stages of the sexual cycle of the bitch by direct examination.*Journal* of *Animal* and *Veterinary Advances*10: 1962-1967.
2. Bergeron L, Nykamp S, Brisson B, Madan P, Sears W. and Gartley C. (2014). Vaginal impedometry for detection of optimal breeding time in bitches. J Am Vet Med Assoc. ;245:1360-6.
3. Bouchard GF, Youngquist RS, Clark B, Concannon P W and Braun WF. 1991. Estrus induction in the bitch using a combination diethylstilbestrol and FSH-P. *Theriogenology* 36(1): 51-65.
4. Christensen, J. (1984) Reproduction in dog and cat. First ed. Bailliere Tindall, London : (1-54)
5. Feldman, E. & R. Nelson, (1996). Canine and Feline Endocrinology and Reproduction. 2nd edn, W.B. Saunders Company, Philadelphia, USA.
6. Goodman M (2001). Ovulation timing: concepts and controversies. Vet Clin North Am Small Anim Pract. 31:219-35.
7. Groppetti D, Aralla M and Bronzo V.(2015). Periovulatory time in the bitch; what's new to know? Comparison between ovarian histology and clinical features. *Animal Reproduction Science* 152: 108-116.
8. Groppetti, D., A. Pecile, C. Barbero & P. Martino, (2012). Vaginal bacterial flora and cytology in proestrus bitches: Role on fertility. *Theriogenology*, 77, 1549–1556.

9. Gupta AK, Dhami AJ and Ghodasra DJ. (2012). Exfoliative Vaginal Cytology: An Effective tool to know the reproductive status of the bitch. *Indian Journal of Field Veterinarians* 8(1): 61-64.
10. Gulavane, S. U. (2001) Studies on the management of fertility and infertility in bitches with special refrence to laproscopy and ultrasonography. Phd thesis submitted to Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.
11. Johnston SD, Root KMVR and Olson PNS. (2001). Canine and Feline Theriogenology, W.B. Saunders Company, Philadelphia, USA p. 16-40.
12. Kusritz MVR. (2012). Practical matters: Vaginal cytology for timing the fertile period in bitch. *A canine reproduction seminar* p. 19-30.
13. Kustriz, M.V.R. (2006). Collection of tissue and culture samples from the canine reproductive tract.Theriogenology, 66: 567-574.
14. Kutzler MA. (2007). Estrus induction and synchronization in canids and felids. Theriogenology, 68(3), 354-74.
15. Moxon R, Batty H, Irons G and England GCW. (2012). Periovulatory changes in the endoscopic appearance of the reproductive tract and teasing behavior in the bitch. *Theriogenology* 78: 1907-1916.
16. Mshelia GD, Amin JD and Chaudhari SUR. (2001). Oestrus detection in the Nigerian Mongrel bitch: Application of vaginal cytology. *Pakistan Veterinary Journal* 21(1): 45-50.
17. Reddy KCS, Raju KGS, Rao KS and Rao KBR. (2011. Vaginal cytology, vaginoscopy and progesterone profile: breeding tools in bitches. *Iraqi Journal of Veterinary Sciences* 25(2): 51-54.
18. Root Kustriz MV. (2006). Collection of tissue and culture samples from canine reproductive tract. *Theriogenology* 66: 567-574.
19. Sharma M and Sharma N. (2016). Vaginal cytology: A historical perspective on its diagnostic use. *Advances in Animal and Veterinary Sciences* 4(6): 283-288.
20. Siregar TN, Melia J, Rohaya TCN, Masyitha D, Wahyuni S, Rosa J, Nurhafni PB and Herrialfian. (2016). Determining Proportion of Exfoliative Vaginal Cell during Various Stages of Estrus Cycle Using Vaginal Cytology Techniques in Aceh Cattle. *Veterinary Medicine International* 1:1-5.
21. Thrall, M. A. and P. N. Olson (1993) In diagnostic cytology of the dog and cat. Ed. R. L. Cowell and R. D. Tyler. American Vet. Publications, Inc., Thornwood Drive : (22-27).
22. Turmalay, L., S. Duro, E. Lika & V. Ceroni, (2011). The hormonal control of estrus in bitches. *Journal of Animal and Veterinary Advances*, 10, 2447–2449.
23. Wehrend A, Von Plato K, GoerickePesch S. (2013). Die exfoliative vaginalzytologie bei der hundin-indikationen, durchfuhrung, interpretation. Tierarztliche Praxis. ;4:267-274.
24. Wright P, Parry B. Cytology of the canine reproductive system. Veterinary Clinics of North America: Small Animal Practice. 1989;19:851-874.
25. Zohara BF, Islam MF, Alam MGS, and Bari FY. (2014). Exfoliative vaginal cytology and serum progesterone during the estrous cycle of indigenous ewes in Bangladesh. *Journal of Embryo Transfer 29*(2): 183-188.



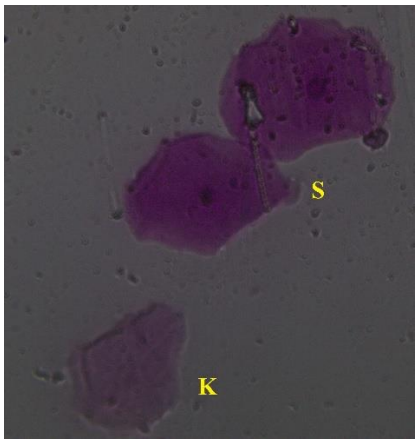


Figure 1, Vaginal cytology of shows non-confirmed cells (P= parabasal cell, I= intermediate cell)

Figure 2, Vaginal cytology of canine shows confirmed cells (S= Superficiial cells cell, K= Keratinised cell)

Table-1 Mean percentage of different vaginal epithelial cells population obtained during stages of reproductive cycles.

Stages of cycle	Parabasal	Intermediate	Superficial	Cornified	Neutrophils	RBCs
Pro estrus	1.88±0.78	52.25±1.38	43.88±1.59	2.00±0.40	+	+++
Estrus	0.00±0.00	5.63±1.26	29.38±3.79	65.00±3.16	+/-	+/-
Diestrus	29.25±0.84	57.25±1.43	4.50±0.40	0.00±0.00	++	+/-
Anestrus	69.50±2.11	28.38±1.90	2.13±0.54	0.00±0.00	+/-	-