

EXFOLIATIVE VAGINAL CYTOLOGY: AN EFFECTIVE TOOL TO KNOW THE REPRODUCTIVE STATUS OF THE BITCH

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(Received 25/04/2012, Accepted 25/06/2012)

ABSTRACT

The exfoliative vaginal cytology was recorded in an attempt to identify the reproductive status/stage of oestrous cycle in 30 bitches. Deep vaginal smears were collected by cotton swab technique from adult bitches in proestrus (n=6), oestrus (n=6), dioestrus/ metoestrus (n=6), anoestrus (n=6) and pregnancy (n=6) and were examined using Giemsa stain. The differential count of exfoliated vaginal epithelial cells showed that the mean percentages of superficial, intermediate and parabasal cells were 65.33 ± 2.38 , 25.17 ± 1.96 and 9.50 ± 0.67 in proestrus, 95.33 ± 0.67 , 4.33 ± 0.62 and 0.33 ± 0.21 in oestrus, 18.33 ± 0.88 , 29.33 ± 1.28 and 52.33 ± 0.72 in dioestrus, 12.33 ± 0.88 , 28.50 ± 1.34 and 59.17 ± 0.95 in anoestrus and 20.50 ± 1.18 , 56.83 ± 3.19 and 22.67 ± 2.60 in pregnant bitches, respectively. Vaginal smears were characterized by presence of massive erythrocytes in proestrus, clear background of smear in oestrus, heavy infiltration of polymorphonuclear cells in dioestrus, very few cells mostly parabasal in anoestrus and dirty background in pregnant bitches. It is thus concluded that vaginal cytology can be conveniently used to evaluate the reproductive stages in bitches.

Key words: Bitches, Exfoliative vaginal cytology, Diagnostic tool, Reproductive stages/status.

INTRODUCTION

The bitches are classified as seasonal monoestrus species. They exhibit 1-2 oestruses in particular periods of the year. This frequency is typically influenced by specific factors like breed, geographical position and management condition which are the most important ones (Christiansen, 1984). The vaginal epithelium of the bitch is characterized by cyclic cellular changes that occur as a result of changing secretory patterns of reproductive hormones, particularly oestrogens. These changes can be determined through exfoliative vaginal cytology (Wright and Parry, 1989). There also exists a relationship between behavioural events and hormonal and physiologic changes in the bitch, although, many bitches have individual variations. It is reported that some bitches show very short period of proestrus, yet they allow for mating and some bitches show very long duration of proestrus/oestrus, even though, they ovulate normally. Therefore vaginal cytology is important to know the exact reproductive stage of the bitch to avoid mating failure, especially in costly breed (Turmalaj et al., 2011). This paper, therefore, describes the vaginal cytologic pattern of the bitches in proestrus, oestrus, dioestrus, anoestrus and pregnancy.

MATERIALS AND METHODS

The study was carried out at Teaching Veterinary Clinical Services Complex, College of Veterinary Science and Animal Husbandry, Anand during 2011-12. A total of 30 deep vaginal smears were prepared from 30 bitches in different stages of reproductive cycle, viz., proestrus, oestrus, dioestrus/ metoestrus, anoestrus and pregnancy, 6 bitches in each group. The samples were collected using cotton swab technique (Aydin et al., 2011). A sterile cotton-tipped swab on wooden stick, 15 cm long was used after moistening with 2-3 drops of sterile saline (0.9%). The lips of vulva were gently separated. The cotton-tipped end of this swab was passed into the dorsal commissure of the vulva.

The swab initially was pressed gently against the caudodorsal surface of the vaginal vault and then advanced in a craniodorsal direction toward the vertebral column, until it passed over the ischial arch into the anterior vagina. The swab was then rotated through a complete revolution in each direction and withdrawn. The cotton tip was rolled from one end of a glass microscope slide to the other (Feldman and Nelson, 1996; Kustriz, 2006). The prepared smears were air-dried, coded and stained with alcoholic Giemsa stain. Stained smears were examined microscopically and interpreted following guidelines of Mshelia et al. (2001).

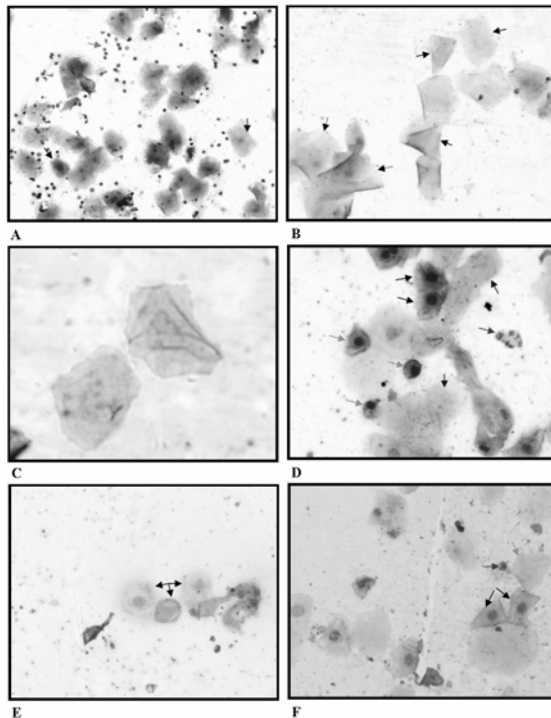


Figure 1: Exfoliative vaginal cytology during different phases of oestrous cycle in bitches

Proestrus: massive erythrocytes (orange arrow), superficial cells (blue arrow) and parabasal cells (black arrow). B. Oestrus: fully cornified anuclear superficial cells (solid arrows) and pyknotic nucleus containing superficial cells (dotted arrows) with clear smear background. C. Oestrus: Magnified view showing fully cornified anuclear superficial cells, flat and angular in shape. D. Dioestrus: intermediate cells (black arrows), superficial cells (blue arrows), parabasal cells (green arrows) and bunch of neutrophils (red arrow). E. Anoestrus: very few cells, arrows showing the parabasal cells. F. Pregnancy: fully superficial cells (green arrow), intermediate cells (black arrow) and parabasal cells (red arrow). Smear background is dirty.

RESULTS AND DISCUSSION

The vaginal epithelium is the one of several target tissues for ovarian hormones. Characteristic changes in exfoliated vaginal epithelial cells occur as a result of changing levels of oestrogens. Changes in the percentage of epithelial cells in vaginal smears can be used to monitor the oestrous cycle in bitches (Johnston et al., 2001). The details of exfoliative vaginal cytology recorded in 30 bitches at different stages of reproductive cycle (6 at each stage) are presented in Table 1 and Plates A to F.

Proestrus

The mean (range) percentages of exfoliated cells, viz., superficial cells, intermediate cells and parabasal cells recorded in bitches at proestrus stage were 65.33 ± 2.38 (range 57-72), 25.17 ± 1.96 (range 19-31) and 9.5 ± 0.67 (range 7-12), respectively. Moreover, the smears were characterized by massive erythrocytes and a very few leucocytes. Background of smears were dirty during early proestrus due to presence of cellular debris and mucin but became clear as the stage approached to oestrus (Plate A). This might be due to increased concentration of estradiol which caused thickening of the vaginal mucosa and proliferation of cell layers. As the mucosa thickened the surface cells changed in size, shape and staining characteristics and became larger, irregularly shaped and ultimately became anuclear. The neutrophils could not enter into the vaginal lumen during proestrus due to thickening of vaginal mucosa, but abundant RBCs could enter into lumen by diapedesis due to estrogenic effect (Reddy et al., 2011).

Oestrus

The mean (range) percentages of superficial,

Table 1. Mean percentage of various types of exfoliative cells seen at different stages of reproductive cycles in bitches

Types of cells	Proestrus (n=6)	Oestrus (n=6)	Dioestrus (n=6)	Anoestrus (n=6)	Pregnant (n=6)
Superficial	65.33 ± 2.38	95.33 ± 0.67	18.33 ± 0.88	12.33 ± 0.88	20.5 ± 1.18
Intermediate	25.17 ± 1.96	4.33 ± 0.62	29.33 ± 1.28	28.5 ± 1.34	56.83 ± 3.19
Parabasal	9.5 ± 0.67	0.33 ± 0.21	52.33 ± 0.72	59.17 ± 0.95	22.67 ± 2.6
Neutrophils	+	+ or -	++	+ or -	+
RBCs	+++	+ or -	+ or -	-	-
Background	Variable	Clear	Variable	Variable	Dirty

intermediate and parabasal cells recorded in bitches at oestrus were 95.33 ± 0.67 (range 93-97), 4.33 ± 0.62 (range 3-6) and 0.33 ± 0.21 (range 0-1), respectively. The smears were characterized by clear background. Neutrophils and erythrocyte were rarely seen (Table 1). This might be due to increased level of blood oestrogen (Plate B, C). Aydin et al. (2011) stated that the duration of oestrus exhibits great variation among bitches and may be quite short in some individuals. Therefore, the determination whether a bitch is in oestrus or not and the identification of the most suitable time for mating are important in terms of reproductive success. The optimum mating time for a bitch occurs when a majority of the epithelial cells (90 %) that are seen on vaginal cytology are superficial cells. Post (1985) suggested that vaginal cytology remains constant in the oestrus and great majority of the cells observed are superficial cells. These cells, also known as cornified cells, have sharp, flat and angular cytoplasmic borders with small pyknotic, fading nuclei or no nuclei.

Dioestrus

The mean percentages of superficial, intermediate and parabasal cells recorded in bitches at dioestrus stage were 18.33 ± 0.88 (range 15-21), 29.33 ± 1.28 (range 25-34) and 52.33 ± 0.72 (range 50-55), respectively. These smears were characterized by drastic decrease in number of superficial cells and marked increase in number of parabasal cells and infiltration of neutrophils in early dioestrus phase (Plate D). This might be due to high level of blood progesterone.

Anoestrus

The mean (range) percentages of superficial, intermediate and parabasal cells recorded in bitches at anoestrus stage were 12.33 ± 0.88 (range 9-15), 28.5 ± 1.34 (range 24-32) and 59.17 ± 0.95 (range 56-62), respectively. The smears were characterized by a very few cells mostly of parabasal and intermediate type (Plate E). Erythrocytes were absent but neutrophils were occasionally seen in some smears.

Pregnancy

The mean percentages of exfoliative superficial, intermediate and parabasal cells recorded in bitches at pregnancy were 20.5 ± 1.18 (range 18-25), 56.83 ± 3.19 (range 44-66) and 22.67 ± 2.6 (range 15-31), respectively. These smears were characterized by drastic decrease in number of superficial cells and marked increase in number of parabasal cells with dirty background (Plate F).

Neutrophils and erythrocytes were rarely seen.

Similar exfoliative vaginal cytological changes in bitches during different stages were observed by Schutte (1967), Mshelia et al. (2001), Ozyurtlu et al. (2006), Aydin et al. (2011) and Reddy et al. (2011). According to Turmalaj et al. (2011) the cytological examination of vaginal smear is one of the important tools in the gynaecological investigations of the bitch, to know any gynaecopathological conditions as well as the stage of cycle and optimum time for hormonal treatment and mating or artificial insemination.

Based on above findings, it is concluded that the exfoliative vaginal cytology is very useful technique to know the reproductive stage of a bitch and to rule out the infertility by knowing the exact time of ovulation or oestrus.

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