VAGINAL CYTOLOGY IN DIFFERENT PHASES OF ESTRUS CYCLE IN BITCH

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The study was conducted on vaginal cytology in different phases of estrus cycle in physiologically normal and healthy bitches of varying breeds between the age group of 2 to 6 years. Vaginal mucous were collected from vagina proper, smears were prepared and stained with Giemsa's stain. Smears studied of were on weekly basis during diestrus and anestrus phases and on alternate day during proestrus and estrus phases and found that during anestrus the parabasal and small intermediate cells were predominant where as the large intermediate, superficial and anucleated cells were significantly ($p<0.05$) low in number. There was a significant ($p<0.05$) increase in large intermediate cells one week before the commencement of proestrus. During proestrus there was a significant increase in large intermediate cells. Superficial and anucleated cells increased towards the end of proestrus. Parabasal and small intermediate cells present in the beginning of proestrus disappeared with the progression of this phase. During estrus the predominant cells were superficial and anucleated; the parabasal and small intermediate cells were scanty, where as large intermediate cells were moderately present. With the end of estrus and commencement of diestrus the parabasal and small intermediate cells reappeared with a significant reduction of superficial and anucleated cells but large intermediate cells were in moderate numbers.

Introduction:

Failure of conception is an important reproductive problem in canines. The most eventful of this conception failure is the improper time of matting (Hewitt and England, 2000) which not only affects the breeding management of a bitch but also result in loss of one more fertile period (Ettinger, 1999). The time of ovulation is variable in bitches in relation to the onset of proestrus. The detection of actual time of ovulation and matting is best determined by hormonal assay which requires a sophisticated well equipped laboratory and costly too. Vaginal cytology, in contrast, is a simple technique that can be used by the practitioners in any simple laboratory setup to help characterize stages of the reproductive cycle of the bitch or to evaluate certain diseases of the genital tract (Valerie *et al.,* 2003) and to determine the definite time of ovulation. The cyclic changes of the endometrium in bitches involving both stromal and glandular compartments have been described but the fate of the surface epithelium after progressive growth and secretion is still unclear (Galabova *et al.,* 2003). Therefore, the present study was undertaken to evaluate the efficacy of vaginal cytological examination for identification of different stages of estrus cycle which may help to identify the proper time of fertile matting.

Materials and Methods

The study was conducted in 14 healthy and fertile bitches of 2-6 years of ages. The bitches of different breeds were maintained in uniform managemental conditions including diet and without matting during the course of study. Vaginal mucous were collected weekly during anoestrus and alternate day during proestrus and estrus from the vagina proper with a sterile non-absorbable cotton swab moistened with normal saline (Edward and Richard, 1996), smears were prepared, fixed with methanol (90%) and stained with Giemsa's solution (Azur-eosin-methylene blue solution, Merck, Bombay). 200 cells at random from different microscopic fields under high-power magnification were counted and typed (Schulte, 1967). Gradation of W.B.C. and R.B.C in the smear was done (Bell and Christie, 1971). The length of proestrus and estrus were variable in different bitches. Therefore, on an average 4 smears during each phase of proestrus and estrus were studied on alternate day basis.

Statistical analysis:

Analysis of variance and critical difference (CD) test were done for different cell types (Snedecor and Cochran, 1981).

Results and Discussion:

A total of 224 vaginal smears were collected, evaluated and results are tabulated for the last 4 weeks of anestrus, proestrus and first 4 weeks of diestrus. The mean values with CD test for all the cell types during the different phases are shown in Table 1A and 1B.

Table 2 illustrates the analysis of variance for all the cell types during the different phases of estrus cycle.

Cytological Features of Vaginal Epithelial Cells:

The vaginal epithelium is influenced by hormonal changes during the estrous cycle, allowing cytologic monitoring of the various reproductive stages (Valerie *et al.,* 2003). The morphologic features of the epithelial cells are described below

Basal Cells: These are the youngest cells of the vaginal epithelium having a round nucleus, small amount of basophilic cytoplasm and serve as precursors of the other epithelial cell.

Parabasal cells: These cells are the smallest vaginal epithelial cells with uniform size and shape and have a round nucleus and basophilic cytoplasm.

Intermediate cells: These cells vary in size but are usually two times the size of parabasal cells. Their borders are round to irregular and folded.

Superficial cells: Superficial cells are the oldest vaginal epithelial cells. They either have small, round, pyknotic nuclei or lack a nucleus if they are cornified. Their cytoplasm is abundant, blue-green, and keratinized.

Significant predominance of parabasal and small intermediate cells during anestrus were recorded (table 1). The parabasal cells decreased in number with subsequent increase in large intermediate cells during early proestrus. WBCs were in moderate to scanty in number/appearance where as RBCs were absent except in the smears few days before the commencement of proestrus as was also observed by Dore (1978), Post (1985), Bell *et al.,* (1971), Griffith and Amoroso (1939).

During proestrus and estrus phase the parabasal and small intermediate cells decreased significantly except during the first few days of proestrus and towards the end of estrus the cells appeared in very less proportions (table 1) simulating with Schulte (1967), Dore (1978) and Post (1985). Prabhakar *et al.,* (1995) signified proestrus by the absence of parabasal cells in the smear and smears in estrus indicated more than 50%of anucleated cells. Large intermediate cells were in moderate numbers. The parabasal and small intermediate cells were absent except at the end when a few cells reappeared in the smear. WBCs were seen for the first few days in scant which disappeared totally through this phase and in estrus also. Erythrocytes were in abundance during proestrus and showed gradual decline with the progression towards estrus. During estrus the presence of RBC varied which corroborated well with the findings of Schulte (1967), Bell and Christie (1971), Prabhakar (1995) and Post (1985).increased plasma concentration of estrogen causing thickening of the vaginal mucosa and an increase in the vaginal cell layers so, the mucosa thickens, the surface cells changes in their size, shape and staining character, becoming larger, irregularly shaped and ultimately aneuclear (England, 1998). Neutrophils at this phase were observed to be very few in number due to estrogen's effect (Feldman and Nelson, 1996). Presence of RBCs might be due to diapedesis through uterine capillaries due to estrogens effect (Olsen *et al.,* 1984).

Diestrus phase began with the reappearance of significant number of parabasal and small intermediate cells. Large intermediate cells were in moderate numbers (table 1). Characteristic phenomenon was the presence of very high number of WBC which appeared with commencement of diestrus. Some of the intermediate cells in-filtered with WBCs named as metestral cells, were also seen in the beginning of diestrus phase as also documented by Schulte (1967), Christie *et al.,* (1972), Bell *et al* (1973), Holst *et al.,* (1974), Dore (1978) and Post (1985).

Parabasal and intermediate cells were the two non-cornified cell types, but superficial cells and anuclear squamous were the two cornified cell types. There was a gradual increase in percent cornification as the bitches progressed from proestrus to estrus, where as there was an abrupt return to complete non-cornification at the onset of diestrus. In proestrus, the vaginal epithelial cell population gradually changed from completely non-cornified to completely cornified. Cornification would be completed at about 2 days before estrogen peaks, about 4 days before standing heat begins. In estrus, the vaginal epithelial cell population would be completely cornified, with greater than 50% of the cells anuclear squamous. On the first day of diestrus, the vaginal epithelial cell population abruptly shifted to complete non-cornification. There might be a large number of polymorphoneuclear leukocytes present, and metestrum cells (non-cornified cells containing leukocytes) and/or foam cells (non-cornified cells containing vacuoles) might be present. Anestrus is a period of reproductive quiescence

with no characteristic physical, behavioral or endocrinologic changes. Vaginal cytology revealed only scanty non-cornified epithelial cells at this stage. (Root Kustritz *et al.,* 2006).

From the above findings it plausibly aid in the successful canine breeding programme through determining the time of mating besides studying the different stages of estrus cycle.

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