ULTRAFAST PAPANICOLAOU STAINING FOR CANINE VAGINAL EXFOLIATIVE CYTOLOGY

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ABSTRACT

Several Staining Technique have been used to evaluate vaginal cell morphology in bitches. The present study was done to evaluate papanicolaou staining to study the vaginal exfoliative cytology in bitches. A total of 500 vaginal smears obtained from bitches presented to the small animal gynaecology unit of Madras Veterinary College Teaching Hospital were utilised for the study. 29.0, 51.8, 8.8 and 5.4, per cent were found to be in proestrus, estrus, diestrus and anestrus. The parabasal, intermediate cells appeared blue and that of superficial and cornified cells pink in colour. This staining technique help to differentiate cell type based on colour rather than morphology which get altered during the collection of vaginal smears within a short time.

Key words: Vaginal exfoliative cytology, Papanocolaou, Canine

In bitches, vaginal exfoliative cytology (VEC) is a well established popular clinical tool to evaluate the changes in cell morphology and staining characteristics occurring during oestrus cycle to optimize the breeding time. Several stains such as Wright's, Diff Quick and New methylene blue have been shown to be excellent to evaluate the cell morphology. However, it is important to choose a stain that could be used to stain the cells of interest, easy to use and relatively inexpensive.

Ultra fast Papanicolaou stain, a modified Papanicolaou stain initially designed to stain fine needle aspiration biopsies for diagnosis of a range of human tumors has been recently evaluated for exfoliative vaginal cytology in bitches (Perez *et al.*, 2005). Hence, in the present study we evaluated the clinical usefulness of ultra fast Papanicolaou stain for VEC in different reproductive conditions in bitches.

Bitches of different breeds and ages presented to the Small Animal Gynaecology out-patient unit of Madras Veterinary Teaching Hospital with the history of genital bleeding were included for this study. Detailed reproductive history was obtained and recorded in all the cases. Accordingly, a total of 500 vaginal smears were collected and examined at the Small Animal Gynaecology Referral unit using Ultra fast Papanicolaou stain to assess the optimum time of breeding and reproductive disease condition. VEC was obtained as per the technique outlined by Feldman and Nelson (1989). Briefly after wiping the vulval area free of dirt, the vulval lips were everted with one hand and with the other, a cotton tipped swab moistened with saline was introduced into the vagina in a cranio-dorsal direction. The swab was rotated once in clockwise and anticlockwise directions and withdrawn (Phemister *et al.*, 1973). The swab tip was gently rolled from one end to the other of a clean grease free glass microscopic slide and air dried.

The smears were immediately stained using the Ultra fast Papanicolaou protocol described by Yang and Alvarez (1995). Briefly, the slide was dipped sequentially

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in normal saline for 30 seconds, one dip in 95% ethanol, 10 seconds in alcoholic formalin, six slow dips in water, 45 seconds in Harris haematoxylin solution, six slow dips in water, six slow dips in 95% ethanol, 1 minute in orange G solution, six slow dips in 95% ethanol and finally six slow dips in 100% ethanol. At least two slides were prepared in each case. Interpretation of all vaginal smears was done by the same clinician I order to avoid bias. The cells were classified from the deepest to the most superficial layer, as parabasal, small and large intermediate, superficial and squamous types. The eosinophilic index (number of vaginal epithelial cells with orange cytoplasm divided by the total number of cells, expressed as percentage) was used to identify keratinized cells (Badinad and Fountbonne, 1993).

In the present study, out of 500 VEC samples analyzed, 145 (29.0%), 259 (51.8%), 44 (8.8%) and 25 (5.4%) were found to be in proestrus, estrus, diestrus and anestrus respectively. The parabasal cell and intermediate cell with basophilic cytoplasm appeared blue and that of superficial and cornified cells with acidophilic cytoplasm pink in colour. This pink colouration is mainly due to the presence of keratohyaline pigment, which is a precursor for keratin (Yang and Alvarez, 1995). Ultra fast Papanicolaou stain is a polychromatic stain which helps to clearly differentiate the superficial and cornified cells (acidophilic) from that of the parabasal and intermediate cells (basophilic). Moreover, the nuclear morphology, like vesiculation (intermediate and superficial intermediate cells) and pyknotic nuclei (superficial cells) are clearly characterized by this stain. In addition, the RBCs which are usually stained during other routine stains are eliminated in the presence of staining.

The counter stains used in this protocol have a high alcoholic concentration which provides cytoplasmic transparency which helps to differentiate cell types based on the colour rather than the morphology, which some times gets altered in the swab technique there by making it a suitable and stable and rapid practical method to be easily adopted.

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