

DETERMINATION OF OVULATION TIMING BY VAGINAL CYTOLOGY USING DIFFERENT STAINS

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The objective of this study was to determine the ovulation timing by vaginal cytology to identifying different stages of oestrus cycle of bitches by using different staining techniques. The present study was conducted to compare of Leishman's and Giemsa stains and analyze the better staining techniques by observing vaginal smears for determine ovulation timing and comparing the characteristics of nucleus, cytoplasm and background staining. Total 24 bitches were selected and two vaginal smears were prepared by using cotton swab and stained with Leishman's and Giemsa stain and total 100 cells were evaluated and cornification index was calculated. The oestrus stages determined with both the stains were consistent with each other. There was non-significant difference between both the stains at different stages of oestrus cycle.

Keywords: Bitches, Cornification index, Oestrus, Staining, Vaginal smear.

One of the important issues in canine reproduction is determination of ovulation timing. The stages of oestrus cycle can be determined by examining clinical, endocrinological and cytological changes (Feldman and Nelson, 1996; Johnston *et al.* 2001). Exfoliative vaginal cytology is widely used for determination of ovulation timing (Wehrend, 2013). Vaginal cytology is preferred as easy and reliable method for determining stages of oestrus cycle It is based on determination of cyclic cellular changes occurring in the vaginal epithelium as a result of reproductive hormone levels, especially estrogens. The increased concentration of estrogen during pro-oestrus and early oestrus periods causing the vaginal walls to thicken as a result of a rapid increase in the number of cell layers in the mucous membrane of the vagina. As a result the cells in the luminal layer of the vagina move away from their blood supply and because they don't have enough blood flow, these cells die and exfoliate into the vaginal fluid (Feldman and Nelson, 1996). The cells are named according

to morphology of the cells (Johnston *et al.* 2001).

Classical staining techniques using stains such as Giemsa, Leishmans, Papanicolaou and New methylene blue are used in vaginal cytology for assessing cellular changes in the microscope. The present study aims at calculation cornification index of vaginal smears stained with Leishman stain and Giemsa stain and studying the characteristics and comparing the cells observed at different stages of oestrus cycle.

Materials and Methods

A comparative study was carried out in 24 bitches each sexually mature, healthy, varying age and breeds. The samples were collected using the cotton swab technique, a sterile cotton swab moistened with few drops of normal saline. The cotton tipped end of the swab was passes into dorsal commissure of the vagina until it passes over the ischial arch. The swab was rotated through a complete revolution in each direction. The tip was rolled on the glass slide to prepare vaginal

smears. Total 48 vaginal smears were prepared. The prepared smear was air dried and one smeared with Leishman's stain for 15-20 minutes. While other smear fixated with methanol and then smeared with Giemsa stain for 30 minutes. Total 100 cells were counted under microscope at 40X and the cytoplasm, nucleus and background and quality of stains were compared to determine ovulation timing. During proestrus parabasal cells and intermediate cells RBC's and other cells like neutrophils are observed while during oestrusanuclear superficial cells and superficial cells.

On Comparison of Leishman's and Giemsa staining in bitches vaginal smears were prepared and stained, different cells such as Anuclear Superficial cells, Superficial cells Intermediate cells and Parabasal cells were classified. Total 100 cells were observed and accordingly Cornification Index was calculated at different stages of oestrus cycle. The stages of sexual cycle determined using Giemsa and Leishman's staining techniques were in total agreement with each other in all samples. It was determined that 11 bitches were in early pro-oestrus while 13 were in late pro-oestrus as also reported by Aydin *et al.*, 2011.

Results and Discussion



Fig.1. Parabasal cells & RBC

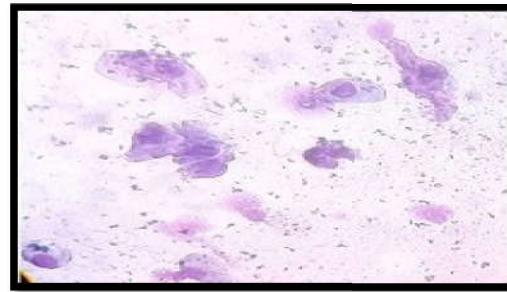


Fig.2. Parabasal cells & Neutrophils



Fig.3. Intermediate cells

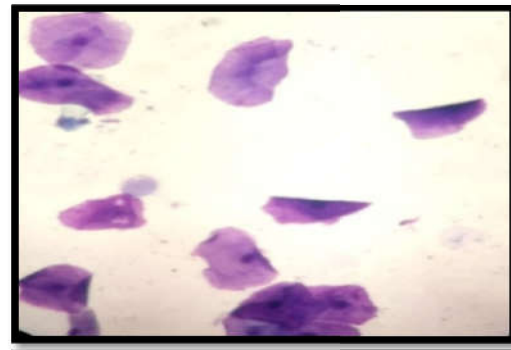
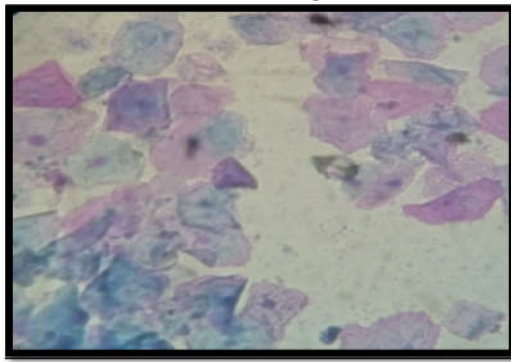
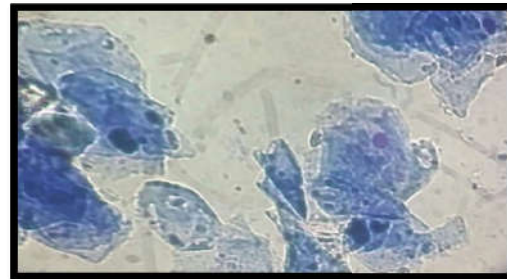


Fig. 4. and 5. Superficial cells

It was observed that by using of both the stains all the cells type i.e. Anuclear superficial cells, Superficial cells, Intermediate cells and Parabasal cells were clearly identified. The Cornification Index, Anuclear Superficial cells percentage,



Superficial cells percentage and Intermediate cells percentage were non-significant ($p > 0.05$) difference between Leishman's and Giemsa stains for different stages i.e. pro-oestrus, oestrus, LH surge and ovulation at 1% level of significance.



Fig. 6. Anuclear superficial cell

Giemsa stain and Leishman's stains both are hematological stains but the time required for processing the vaginal smear was lesser in Leishman's stain on the other hand Giemsa stain requires 30 minutes therefore it is time consuming. For Leishman's stain only one solution is required. Only one step was required for Leishman's staining while Giemsa staining required fixation of smear and multiple steps. Leishman's stain could be used on the spot to process the sample with no expensive instruments. It was cost effective with less amount of reagents required. Therefore, it could be concluded that Leishman's staining technique can be use more efficiently for determination of ovulation timing in bitches. In this study, on comparison of Leishman's stain and Giemsa stain of vaginal cytology for ovulation detection timing, it can be concluded that Leishman's stain is more economical, less time consuming and easy to use.

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