

MANAGEMENT OF INFERTILITY IN BITCHES USING EXFOLIATIVE VAGINAL CYTOLOGY

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The bitch is monoestrous with a long pro-oestrus and oestrus both averaging about nine days each. The duration of pro-oestrus and oestrus might be as short as two to three days or may persist as long as three weeks (Concannon *et al.*, 1989). This makes the correct timing of mating difficult for optimum conception. The popular practice is to allow one or two matings after cessation of pro-oestral bleeding or after nine days from the onset of pro-oestrus. However, poor fertility is obtained in bitches with aberrant pro-oestrus and oestrus.

As a means of determining different stages of canine oestrous cycle and to assess the best time to breed a bitch, exfoliative vaginal cytology, has been reported to be very reliable. Schutte (1967) suggested certain indices like eosinophilic index, karyopyknotic index and superficial cell index to arrive at the best time for mating bitches. Olson and Husted (1986) recommended breeding a bitch when 90 per cent or more of epithelial cells are of superficial type. England (1992) demonstrated that breeding a bitch based upon the peak of anuclear cell index offers advantages over other methods to improve the breeding management of bitches. The present study was undertaken to evaluate the anuclear cell index as a method for improving fertility management in bitches.

Materials and methods

Bitches brought to the University Veterinary Hospital, Kokkalai, Thrissur which did not conceive on previous two or more matings formed the material for the study. Vaginal smears were collected on alternate days from the first day of pro-oestrus to the beginning of dioestrus as evidenced by the reappearance of large number of parabasal and intermediate cells (Holst and Phemister, 1974). Vaginal fluid was collected using an adaptation of the technique by Allen and Dagnall (1982). A sterile glass pipette with a rubber bulb at the distal end was introduced into the vagina, carefully directing the pipette upwards to the vestibule and at the vestibulo-vaginal junction the pipette was redirected cranially upto the anterior vagina. Vaginal fluid was collected, thin smears were prepared and air dried.

The vaginal smears were stained by Wright-Giemsa stain and examined under high power. The epithelial cells were classified into nine types according to Christie *et al.* (1972). The anuclear cell index was arrived at by counting cell types 1, 2 and 3.

The bitches were mated with known fertile males two days after the peak of anuclear cells and there after on alternate days to a maximum of three matings. Pregnancy diagnosis was carried out by abdominal

palpation between 24 and 30 days of mating. The pregnant bitches were followed up until whelping. The day of plasma LH surge was calculated retrospectively on the assumption that it would have occurred 65 ± 1 days prior to the day of whelping (Concannon *et al.*, 1983).

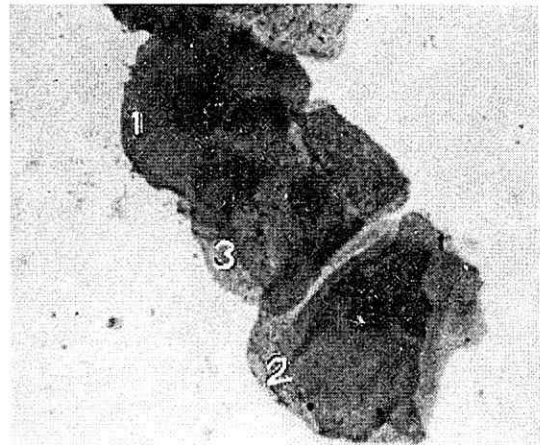
Results and discussion

In four of the six bitches only one anuclear cell peak was recorded between the ninth and fifteenth day (mean 12 days) from the onset of pro-oestrus. On the other hand in the other two bitches two anuclear cell peaks were noticed with the first peak on the seventh and ninth day (mean eight days) respectively and the second peak on the thirteenth day from the onset of pro-oestrus. Maximum anuclear cell peaks were between 51 and 83 per cent.

Four of the six infertile bitches (66.7%) mated on the basis of anuclear cell peak became pregnant. They were mated 2.5 ± 0.5 (SD) times. The mean litter size was 7.3 ± 1.9 (SD). There was no significant difference in the day of anuclear cell peaks between the bitches which became pregnant on mating and those which did not conceive.

The calculated day of LH peak was five to twelve (mean 8.8) days after the onset of pro-oestrus. For the bitches in which one anuclear cell peak was identified, the interval between the calculated day of LH surge and the anuclear cell peak was one to four (mean 2.3) days. Of the two bitches with two anuclear cell peaks, one whelped. The interval between the calculated day of LH surge and the first anuclear cell peak was 2 days and the interval between the calculated day of LH surge and the second anuclear cell

peak was four days (Fig.). The interval from the calculated LH peak and onset of metoestrus was recorded to be 10.3 ± 2.1 (SD) days. It was reported that the interval from the LH peak to ovulation and subsequent maturation of oocytes was about four to five days (Phemister *et al.*, 1973., Concannon *et al.*, 1977., Tsutsui, 1989). The fertile life span of the sperm in the reproductive tract of the bitch is five to six days (Doak *et al.*, 1967., Concannon *et al.*, 1983). Thus it appeared that the ideal time for mating for optimal conception is two days after the observation of the first anuclear cell peak, and thereafter two or three matings on alternate days.



In two of the bitches which became pregnant, erythrocytes were present in the vaginal smear throughout the mating period. In one of them the reddish discharge continued for three more days after the last mating. In both these cases, there was failure of conception in previous matings probably because these bitches might have mated only after cessation of bleeding.

Summary

The oestrous cycle of six bitches which did not conceive on previous two or more

matings were monitored using exfoliative vaginal cytology. Four of the six bitches had one anuclear cell peak and the remaining two bitches had two anuclear cell peaks. Maximum anuclear cell peaks ranged from 51 and 83 per cent. When they were mated on the basis of anuclear cell peaks, four of the six bitches whelped with a mean litter size of 7.3 ± 1.9 (SD) pups. LH peaks were calculated retrospectively in all bitches after whelping. Based on the results of the present study it is recommended that bitches be bred two days after first anuclear cell peak and thereafter on alternate days (maximum two or three matings) for optimal fertility.

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