

VAGINAL EXFOLIATIVE CYTOLOGY, SERUM PROGESTERONE AND VAGINOSCOPY FOR BREEDING MANAGEMENT IN BITCHES

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The present study was undertaken to record the conception rate in bitches following breeding during the fertile period, determined based on vaginal exfoliative cytology, serum progesterone profile and vaginoscopy. Thirty six apparently healthy animals were randomly assigned to three equal sized groups of 12 animals each. In Group I, bitches were made to breed based on vaginal exfoliative cytology (80 % cornification). In Group II, bitches were recommended to breed based on vaginal cytology and serum progesterone concentration (4 ng/ml). In Group III, bitches were advised to breed based on vaginal cytology, serum progesterone concentration and vaginoscopic observations of vaginal mucosa (Crenulation score 1 or 2). Conception rate of 16.67 per cent (2/12), 33.33 per cent (4/12) and 91.67 per cent (11/12), respectively was found for bitches in Group I, II, and III. Conception rate in Group III was statistically significant ($p < 0.05$) in comparison with Group I and II. It is observed that use of combination of methods to predict breeding time yields better conception rate than use of any one method.

Keywords: Breeding time, Conception rate, Cytology, Vaginal exfoliative cytology, Vaginoscopy.

One of the important components of successful reproduction in canine is the breeding management. The duration of the estrous cycle and the individual stages of the estrous cycle vary both between bitches and between cycles in the same bitch. Despite the existing variability matings are done on standard number of days, usually day 9, from the start of vulval bleeding which is unscientific and leads to apparent infertility. Therefore, accurate monitoring and timing of the cycle is essential to achieve a fertile mating. Optimal fertility can be obtained if a bitch is mated two to four days after ovulation and techniques used for breeding management include vaginal exfoliative cytology, vaginoscopy and plasma/serum progesterone (P_4) concentrations (Jeffcoate and Lindsay, 1989; Hewitt and England, 2000). Several studies suggest the breeding to commence when the vaginal cornification reaches 80 per cent or more and continue thereafter till there was abrupt decline in the percentage of cornified cells (Johnston *et al.*, 2001; Srinivas *et al.*, 2004). Serum progesterone concentration has been reported to increase gradually from basal values (0.5 to 1.0 ng/ml) with the advancement of proestrus

and exhibit a rapid increase (1.0 to 3.0 ng/ml) around the time of LH surge (England and Concannon, 2002). Identification of this rapid rise allows an indirect method of predicting the LH surge, and therefore, the fertile period of the bitch. Observation of progressive changes in vaginal mucosa via vaginoscopy provides an indication of the stage of the estrous cycle and ovulation timing (Lulich, 2006). The present study was taken up to assess the optimum breeding time in dogs by using combination of methods and to assess the conception rate.

Materials and Methods

This study was conducted on bitches presented to the Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bengaluru for determination of mating dates. The examined population consisted of 36 apparently healthy bitches from different breeds and of different age (ranged from 1.5 to 7 years). On presentation, history about the reproductive status of the bitches was obtained. General physical examination and clinical examination of the reproductive tract was carried out. The presence of behavioural signs of estrus, teasing reflex, relaxations of vulva and colour

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of vaginal discharges were noted at the time of examination.

Thirty six animals were randomly assigned to three equal sized groups of 12 animals each (n=12). Group I bitches were recommended to breed on the basis of vaginal exfoliative cytology (VEC). The vaginal fluid for the study of exfoliative cytology was collected using aspiration technique and smears were stained using Giemsa stain. Exfoliative cytology was studied every other day until the percentage of superficial cells reached a minimum of 80 per cent and above. If the vaginal cornification was between 80 per cent and 90 per cent first mating was recommended two days later and two subsequent matings on alternate days. If the superficial cell percentage was 90, first mating was recommended on the same day and two subsequent matings on alternate-day basis.

In Group II, bitches were advised to breed based on VEC and serum progesterone concentration. Blood samples for estimation of serum progesterone were collected on the day of presentation of the animal for VEC. Serum progesterone concentration was estimated by using progesterone RIA kit. Bitches in this Group were recommended to breed when the vaginal cornification was 80 per cent and serum P₄ concentration was 4 ng/mL. Based on these, three matings were suggested on alternate days.

In Group III breeding was advised based on VEC, serum progesterone

concentration and changes in the vaginal mucus membrane as observed through vaginoscope. The vaginoscopic examination was performed when the animals were presented for VEC examination. The vaginoscopic equipment consists of a rigid endoscope of 15 cm length with an oblique viewing angle of 30° was used for the study. Vaginoscopic assessment was done based on the size of the mucosal folds, colour and on the character and colour of any fluid present. Interpretation of the endoscopic appearance was done based on the modified version of Jeffcoate and Lindsay's vaginal mucosal scoring (VMS) system.

Changing appearance of vaginal mucosa was assigned as a crenulation score on a scale from 0 to 3:

0 = pale red oedematous mucosa (expected prior to the preovulatory LH peak) (Fig. 1a)

1 = shrinking pale mucosa without angulation (expected at the preovulatory LH peak) (Fig. 1b)

2 = shrinking pale mucosa with further angulation (expected at ovulation) (Fig. 1c)

3 = maximal wrinkling and pale mucosa (expected at oocyte maturation) (Fig. 1d)

Bitches in Group III were advised to breed when vaginal cornification was 80 per cent, serum P₄ concentration was 4 ng/mL and on the day when shrinkage and angulations of mucosal profile observed through vaginoscope (Crenulation score 1 or 2). And then mating was repeated at two-day intervals for two times.



Fig. 1a. Vaginoscopic view - pale red oedematous mucosa scored as 0



Fig. 1b. Shrinking pale mucosa without angulation scored as 1



Fig. 1c. Shrinking pale mucosa with further angulation scored as 2

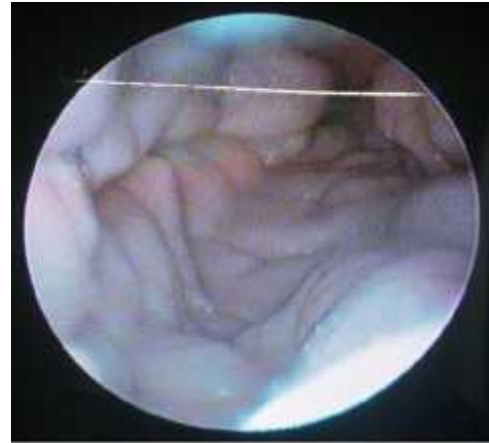


Fig. 1d. Maximal wrinkling and pale mucosa scored as 3

All the animals which were recommended for mating using different methods were subjected to pregnancy diagnosis at 30 to 35 days later by abdominal palpation or ultrasonography examination. Animals diagnosed pregnant were followed till completion of pregnancy period and information on the litter size was also obtained after whelping.

The data was expressed as mean \pm S.E. Chi-square test was performed to compare the conception rate in all the groups using Graph Pad Prism trial version 5.01, 2007. All significant differences were set at $P < 0.05$.

Results and Discussion

In this study, we found that conception rate in bitches mated on the basis of vaginal cytology only (80 per cent cornification), as diagnosed by abdominal palpation and ultrasound examination on day 30 after the last mating was 16.67 per cent (Table. 1). Similarly Okkens *et al.* (1985) reported 30 per cent conception rate when vaginal cytology was used as the sole method to detect ovulation. Contrary to us a significantly higher conception rate of 92 per cent was reported by England (1992) who considered ferning pattern of vaginal fluid in addition to cornification index to recommend the mating dates for bitches. Low conception rate in this study may be due to the factors like the male used, nutrition and non-compliance of owners for date of mating.

There has been controversy regarding the usefulness of vaginal cytology and it has been reported that the changes in cell type in the vaginal smear are too variable to reflect the underlying endocrinological events. This study confirms that vaginal cytology is of use for the detection of proestrus and estrus; is not an accurate predictor of the time of ovulation which is in agreement with the findings of Wright (1990) who stated that the time of occurrence of ovulation is variable (3 to 20 days) with respect to the onset of proestrus and the attainment of maximum vaginal cornification (2 to 13 days after maximum cornification). Therefore, vaginal cytological evaluation is ultimately a retrospective method of monitoring periovulatory events in bitches as also reported by Levy and Fontbonne (2007).

The conception rate following mating on the basis of vaginal exfoliative cytology and serum progesterone concentration was 33.33 per cent (Table-1). Further, following recommendation of mating based on VEC, serum progesterone profile and vaginoscopy, 11 out of 12 bitches (91.67%) were diagnosed to be pregnant and conception rate (Group III) was statistically significant ($P < 0.05$) in comparison with bitches of Group I and II (Table. 1). Increased conception rate in the present study might be due to increased accuracy of examination by using combination of methods. Several authors inferred that vaginoscopy is highly useful for predicting the optimum breeding time as this technique allows us to track the effects of

both estrogen and progesterone. Crenulation (wrinkling) of the vaginal mucosa is more reflective of the occurrence of the preovulatory LH peak and ovulation than any

changes in the vaginal smear at that time, as vaginal smears are inconsistent as also reported by Lindsay and Concannon (1986) and Jeffcoate and Lindsay (1989).

Table-1. Conception rate in Various Groups

Groups	No. of bitches pregnant	No. of bitches non-pregnant	Conception rate (%)
I (n=12)	2	10	16.67*
II (n=12)	4	8	33.33*
III (n=12)	11	1	91.67

* Significant difference with Group III (P < 0.05) X² = 14.93

From the results of this study we can conclude that to determine the optimum breeding time one single symptom or examination was not sufficient; the diagnosis should be based on combination of methods rather than based on one method. Fertilization period can be best determined using a combination of vaginal cytology, estimation of serum P₄ concentration and vaginoscopic observations of vaginal mucosa.

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