VAGINOSCOPIC DETERMINATION OF OVULATION AND FERTILE BREEDING TIME TO OPTIMIZE CONCEPTION IN BITCHES

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ABSTRACT

Twenty sub fertile bitches of different breeds at day 1 of proestrual bleeding were subjected to vaginoscopy and LH estimation to predict the time of ovulation and fertile breeding time. Based on vaginoscopic assessment of vaginal mucosa, the mean length of shrinkage period without angulation corresponding to the period of LH surge and ovulation was 5.45 ± 0.21 days while, the mean length of the angulated shrinkage period corresponding to fertilization period was 5.00 ± 0.35 days. The LH surge occurred on an average 9.95 ± 0.67 days from day 1 of proestrual bleeding and the calculated time of ovulation (LH surge + 48 h) was 11.95 ± 0.67 days from the onset of proestrual bleeding and ranged from 9 to 20 of the cycle. A high co-efficient of correlation ($P < 0.01$) of 0.96 was observed between LH and vaginoscopy.

KEY WORDS: Ovulation, Vaginoscopy, LH surge, Bitches

INTRODUCTION

The fundamental problem in programming successful breeding schedule and in investigation of infertility is the significant variation between bitches, within cycles of the same bitch, and in the duration of the critical phases of proestrus and estrus. The optimal time of mating or insemination is just before the time the ova can be fertilized. Since artificial insemination with frozen semen is becoming popularized among canines (Hase *et al.*, 2000) a greater precision of estimating the time of ovulation is required due to poor post-thaw viability of sperm. Frozen thawed semen has an estimated intra-uterine fertile life span of less than 24 hours (Concannon and Battista, 1989) when compared to fresh semen, which has a fertile intra uterine life span of more than 4 days (Concannon *et al.*, 1983). Thus the development of a rapid and reliable method for predicting ovulation in dogs would have a large impact on canine reproduction. Such a method would improve the fertility and also the cost efficiency of artificial insemination techniques (cooling and freezing). It would also find application in breeding and infertility management and in diagnostic research and embryo transfer in canines (Bouchard *et al.*, 1991). The present study was taken up to study the use of vaginoscopy in determination of ovulation and fertile breeding time in bitches.

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MATERIALS AND METHODS

Twenty healthy sub fertile bitches of different breeds in proestrus presented to the Small Animal Obstetrics and Gynaecology ward of Madras Veterinary College formed the animals for clinical study. Vaginoscopy was performed in these bitches at intervals of two days starting from day 5 of proestrus till day 1 of diestrus (as determined by vaginal cytology) using a rigid fibre optic endoscope (Karl Storz, GmbH, Tiuttlingen, Germany). The sterilized endoscope was introduced into the vagina in a craniodorsal direction after everting the vulval lips. The instrument was inserted as far cranially as it passed freely and the vaginal mucosa was examined for colour, texture and shape of the mucosal folds as the instrument was withdrawn 1 to 4 cm and then slowly advanced to the original position. Any obstruction of the view due to moisture,

mucus or debris at the scope's tip was resolved by withdrawing the scope, rinsing and then reinserting it. Slight insufflation induced during observation aided passage of the scope and to get a more panoramic view.

The changes in the mucosal fold contours and profile, in mucosal color and in the character and color of any fluid present, was assessed vaginoscopically and vaginal mucosal scores (Table 1) were assigned to each of these changes based on the scoring pattern of Lindsay (1983). The changes in the vaginal mucosa were then correlated with the occurrence of LH peak in the twenty bitches studied

TABLE 1. VAGINOSCOPIC MUCOSAL SCORE (VMS) SYSTEM

Period	Letter Denoting Character of Period	Score and Range
Edematous	O	O- Edematous
Shrinkage without angulation of mucosal profiles (Non Δ)	S	S1 - Onset of Shrinkage. S2 - Obvious Shrinkage S2a - Increased to maximum Shrinkage.
Shrinkage with angulation of mucosal profiles (Δ)	S	S3 - Onset of angulation S4 - Obvious angulation S5 - Increased to maximum angulation.
Declining Shrinkage and rounding out of mucosal profiles	D	D5 - On set, extreme angulation with thinning of mucosa D3 - Initial rounding out D1 - Obvious rounding out
Diestrus onset	Do	Do

Daily blood samples were collected from the cephalic vein in heparinized tubes starting from day 7 of proestrus until day I of diestrus (as determined by vaginal exfoliative cytology). The plasma obtained by centrifugation was kept stored at -80° C for the LH estimation.

Plasma LH concentrations were determined using a double antibody radioimmunoassay as described by Miller and Aehnelt (1977) using ovine LH antiserum (NIDDK - anti - OLH - 1) and purified ovine LH (NIDDK - OLH - 1 -4) both for iodination and for use as reference standard (Provided by NIDDK, Bethesda, MD, USA).

RESULTS AND DISCUSSION

LH levels rose from below detectable levels to around 10.4 ± 5.02 ng/ml one day before the surge to reach peak levels of 166 ± 12.06 ng/ml (80 to 260 ng/ml) during the period of surge and thereafter fell to non-detectable levels 2 days later. Peak levels of 166 ± 12.06 ng/ml obtained in the present study were within the range reported by Olson et al. (1982) (80 to 749 ng/ml, mean 402 ng/ml). LH surge occurred on an average 9.95 ± 0.67 (7 to 18) days from day 1 of proestrual bleeding and was in accordance with the findings of England (1992). The calculated time of ovulation in relation to LH surge (LH surge + 48 h) in the present study was 11.95 ± 0.67 days from onset of proestrual bleeding and ranged from 9 to 20 days which was in accordance with the findings of Johnston (1980).

On vaginoscopic examination, the period of proestrus was characterized by oedematous mucosal folds with rounded profiles as rising plasma oestradiol concentration promoted tissue fluid retention in the mucosa and proliferation of its epithelial layers. The colour of the mucosa varied from pink to cream white. The serosanguinous uterine discharge appeared as reddish fluid in between the furrows of mucosal folds (Plate 1). Late proestrus or early estrus was

characterized by shrinkage of vaginal mucosa without angulation (Non AD) which was related to decreasing concentration of oestradiol and consequent withdrawal of fluid retention. Progression to a more obvious shrinkage without angulation corresponded to the preovulatory LH surge period and ovulation (Plate 2). This was then followed by the period of shrinkage with angulation (AD) which corresponded to the fertilization period in bitches. During this period, the folds became increasingly sharp in profile (Plate 3).

Onset of mucosal shrinkage without angulation occurred between -3 and 0 days of LH surge while obvious mucosal shrinkage without angulations occurred between days 0 to 2 after LH surge. Onset of shrinkage with angulation, which corresponded to fertilization period, occurred 3 to 6 days after LH surge.

The mean length of the proliferative edematous period corresponding to the early preovulatory phase was 7.15 ± 0.68 days with a range of 4 to 14 days. The mean length of the non-angulated shrinkage period corresponding to the periovulatory phase was 5.45 ± 0.21 days with a range of 4 to 7. The mean length of the angulated shrinkage period corresponding to the fertile period was 5.00 ± 0.35 days with a range of 2 to 8.

The period of VMS S3 to S5 fell within the period of standing estrus and, between LH peak and the estimates of time required for completion of ovulation, ova maturation and fertilization period. VMS S4 and S5 were striking vaginoscopic features and therefore were useful indicators of peak fertility. The transition from VMS S1 to S2 correlated well with LH peak and occurred on the day of LH surge or 1 to 2 days after the LH surge. Natural mating or artificial insemination was recommended at VMS S4 and S5 which coincided with fertilization period. Lindsay (1983) reported that high fertilization rates could be achieved from single matings of bitches with VMS S4 or S5, which consistently fell within peak fertilization period.

Selection of the angulated period for natural mating is particularly important when for management reasons the fertility of the dog or bitch is unknown or suspected. When only a single mating is possible, peak fertility is most likely to be achieved by delaying mating until the peak AD period. While using frozen semen, insemination should be performed in peak fertilization stages S4 and S5 in order to maximize conception rates as frozen semen has very short post thaw sperm viability.

Since, plasma LH surge is more closely associated with ovulation, the calculated time of ovulation based on LH assay was taken as the standard in the present study and the time of ovulation based on vaginoscopy was compared with it. A high coefficient of correlation ($P < 0.01$) of 0.96 was observed between LH and vaginoscopy indicating that the technique was highly useful for predicting the optimum breeding time in bitches within a clinical practice situation.

Plate 1. Edematous proliferative period (Early proestrus).

Plate 2. Non angulated shrinkage period.

Plate 3. Angulated shrinkage period.

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