Vaginal cytology, vaginoscopy and progesterone profile: breeding tools in bitches

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Abstract

The exfoliative vaginal cytology, vaginoscopic examination of vaginal mucosa and progesterone profiles were recorded in an attempt to identify the ideal time of breeding in bitches. A total of 18 anestrus bitches were selected and divided into 03 groups (Control, CABG and eCG groups). The bitches in control group did not receive any treatment and exhibited estrus. The estrus was induced with Cabergoline (CABG) and equine Chorionic Gonadotropin (eCG) in the other two groups of bitches. In control group, higher percentage of superficial cells (89.94 ± 0.64) and lower percentage of intermediate (7.30 ± 0.77) and parabasal cells (2.76 ± 0.30) were characteristic vaginal cytological changes during estrus. Vaginoscopic examination of CABG group of bitches revealed that the vaginal mucus was creamy and paper white with angular shrinkage during estrus. In eCG group of bitches, the plasma progesterone concentration was 1.55 ± 0.19 ng/ml on day 8.00 ± 0.71 of proestrus. The conception rates were 66.66, 83.33 and 83.33 per cent in Control, Cabergoline and eCG groups, respectively. The litter size varied from 3.50 ± 1.12 to 4.83 ± 0.83 in the three groups.

Keywords: Bitches; Estrus; Exfolaitive; Vaginal Cytology; Vaginoscopy; Progesterone.
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المنظار المهبلي والتغيرات الخلوية لسطح المهبل وتركيز هورمون البروجسترون: معطيات التناسل عند إناث الكلاب

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الخلاصة

في محاولة لتعيين الوقت المناسب للتناسل لدى اناث الكلاب، تم إجراء الفحوصات بالمنظار المهبلي، والتغيرات الخلوية لسطح المهبل وقياس تركيز هورمون البروجسترون بالدم. تم اختيار ١٨ كلبة في حالة عدم شبق لأكثر من ١٨٠ يوما وقسمت الى ثلاث مجاميع (ست كلبات لكل مجموعة). المجموعة الأولى: مجموعة السيطرة التي لم تخضع لأي علاج حتى ظهور الشبق. المجموعة الثانية: عولجت باستخدام ال (Cabergoline). أما المجموعة الثالثة فقد عولجت باستخدام الهورمون المشيمي الخيلي (eCG). ومن خلال الفحص بالمنظار المهبلي للمجموعة الثانية تبين ان مخاط المهبل حليبي قشداني المظهر و ورقي أبيض مع انكماش ذو زوايا واضحة خلال فترة الشبق. وأظهرت مجموعة السيطرة (الأولى) تغيرات مميزة لخلايا سطح المهبل خلال فترة الشبق حيث لوحظت نسبة عالية من الخلايا السطحية (٨٩,٩٤ ± ٠,٦٤) ونسبة أقل من الخلايا الوسطية (٠٧,٣٠ ± ٠,٧٧) وكذلك الخلايا جنب الأساسية (٢,٧٦ ± ٠,٣٠). أما المجموعة الثالثة فقد كان تركيز البروجسترون بالدم ١,٥٥ ± ٠,١٩ نانوغرام / مل في اليوم ٨,٠٠ ± ٠,٧١ من فترة ما قبل الشبق. كانت نسب الإخصاب ٦٦,٦٦% ، ٨٣,٣٣% و ٨٣,٣٣% للمجاميع الأولى والثانية والثالثة على التوالي. واختلف عدد المواليد بين ٣,٥٠ ± ٠١,١٢ الى ٤,٨٣ ±٨٣,٠ في المجاميع الثلاثة.

Introduction

There is a considerable variation in the time of ovulation in relation to the onset of serosanguinous discharge of early proestrus in bitches. This is often not understood by dog breeders and they frequently impose standard mating regimes upon their bitches. These regimes usually involve a plan for breeding at a predetermined or defined number of days after the onset of vulval "bleeding". As a result of this, many bitches are often mated at an inappropriate time, and this constitutes the commonest cause of apparent infertility in bitches (1). There are several investigative methods for identifying the optimal mating time including examination of exfoliated vaginal cells, vaginal endoscopy and measurement of plasma progesterone concentrations.

Vaginal cytology is a simple technique that can be used by practitioners to characterize stages of the reproductive cycle of the bitch (2). Vaginoscopy is a useful diagnostic procedure for evaluating the nature and extent of changes in vaginal mucosa (3). Hence the present study is aimed to suggest breeding time based on vaginal cytology, vaginoscopy and progesterone profile.

Materials and methods

Eighteen bitches of Labrador and German shepherd breeds in anestrus stage were utilized for the study. The bitches were assigned randomly to three groups so that each group consisted of six bitches. The bitches, which did not exhibit estrus for more than 180 days were considered as 'anestrus' condition. Control Group: Six bitches were utilized as untreated controls. CABG Group: Six bitches were subjected to induction of estrus by using Cabergoline (Cabgol, Sun Pharmaceutical Industries, Mumbai, India) at a dose of 05 µg / kg body weight orally (administered once daily) until the detection of proestrual bleeding. eCG Group: Six anestrus bitches were subjected to induction of estrus with the eCG (Folligon- Intervet International, Boxmeer, Netherland) at a dose of 20 IU/kg body weight I/M for 05 consecutive days and on day 05 injected with hCG (Chorulon - Intervet International, Boxmeer, Netherland) at a dose of 500 IU I/M.

The vaginal smears were collected every alternate day from control whenever the bitches entered into proestrus till the day of diestrus for the purpose of determining the stage of estrous cycle. The vaginal smears were stained with geimsa stain (4, 5).

The bitches which responded to Cabergoline treatment were subjected to vaginoscopic examinations at 02 days interval starting from day 0 of proestrus till first day of diestrus / pregnancy. The change in the mucosal colour, mucosal contours and profiles in the mucosal folds were recorded vaginoscopically (6).

In eCG treated bitches, the plasma was collected on day 1 of proestrus and thereafter every alternate day till early estrus. The progesterone profile was estimated by ELISA method (United Biotech Inc, 110 Pioneer ways, California).

The estrus bitches in control group were mated as per the vaginal cytology (5) whenever the 80 percent cornified epithelial cells appeared and there after 3rd day. The bitches which responded to Cabergoline treatment were mated whenever the shrinkage of vaginal mucosa with angulation (7) was observed and thereafter 3rd day. The bitches responded for eCG were mated whenever the progesterone profile was more than 1 ng/ml thereafter 3rd day. The pregnancy was diagnosed by ultrasonography after 30 days of mating.

The data was subjected to statistical analysis (analysis of variance – ANOVA) with Microsoft excel 2003 version.

Results and discussion

The vaginal smears collected from the control bitches revealed that the superficial cells appeared in higher per cent during proestrus (65.30 ± 1.40) which was further increased to still higher per cent during estrus (89.94 ± 0.63). Thereafter the superficial cells decreased in numbers during diestrous (18.52 ± 0.94) and anestrous (12 ± 0.70) phases of estrous cycle. The per cent of intermediate cells was observed in almost equal number during proestrous (25.17± 1.17), diestrous (29.77 ± 1.98) and anestrous (28.38 ± 1.44) phases of estrous cycle. But these cells were very less in number (7.30 ± 0.77) in the bitches during the estrous phase of the study. The parabasal cells were predominantly noticed during diestrus (51.71 ± 1.05) and anoestrus (58.92 ± 1.37 percent). They appeared occasionally during proestrous (9.53 ± 0.29) and estrous (2.76 ± 0.30) phases of the estrous cycle (Figure 1).

Similar exfoliative vaginal cytological changes in bitches during proestrus were observed by (2,4,8,9). This might be due to increased concentration of estradiol which caused thickening of the vaginal mucosa and proliferation of cell layers. As the mucosa thickened the surface cells changed in size, shape and staining characteristics and became larger, irregularly shaped and ultimately anuclear (10). The neutrophils could not enter into the vaginal lumen during proestrus due to thickening of vaginal mucosa, but abundant RBCs could enter into lumen by diapedesis due to estrogenic effect (5). During diestrus, the progesterone levels remained at high concentration resulting in sloughing of vaginal epithelium. The number of cell layers decreased, deeper cells were uncovered and as a result, the per cent of anuclear cells decreased (10), with higher numbers of neutrophils as noticed (5). The background on the vaginal smears appeared dirty during proestrus because of the presence of cellular debris and mucin as noticed by Feldman and Nelson (5). But during estrus, the background

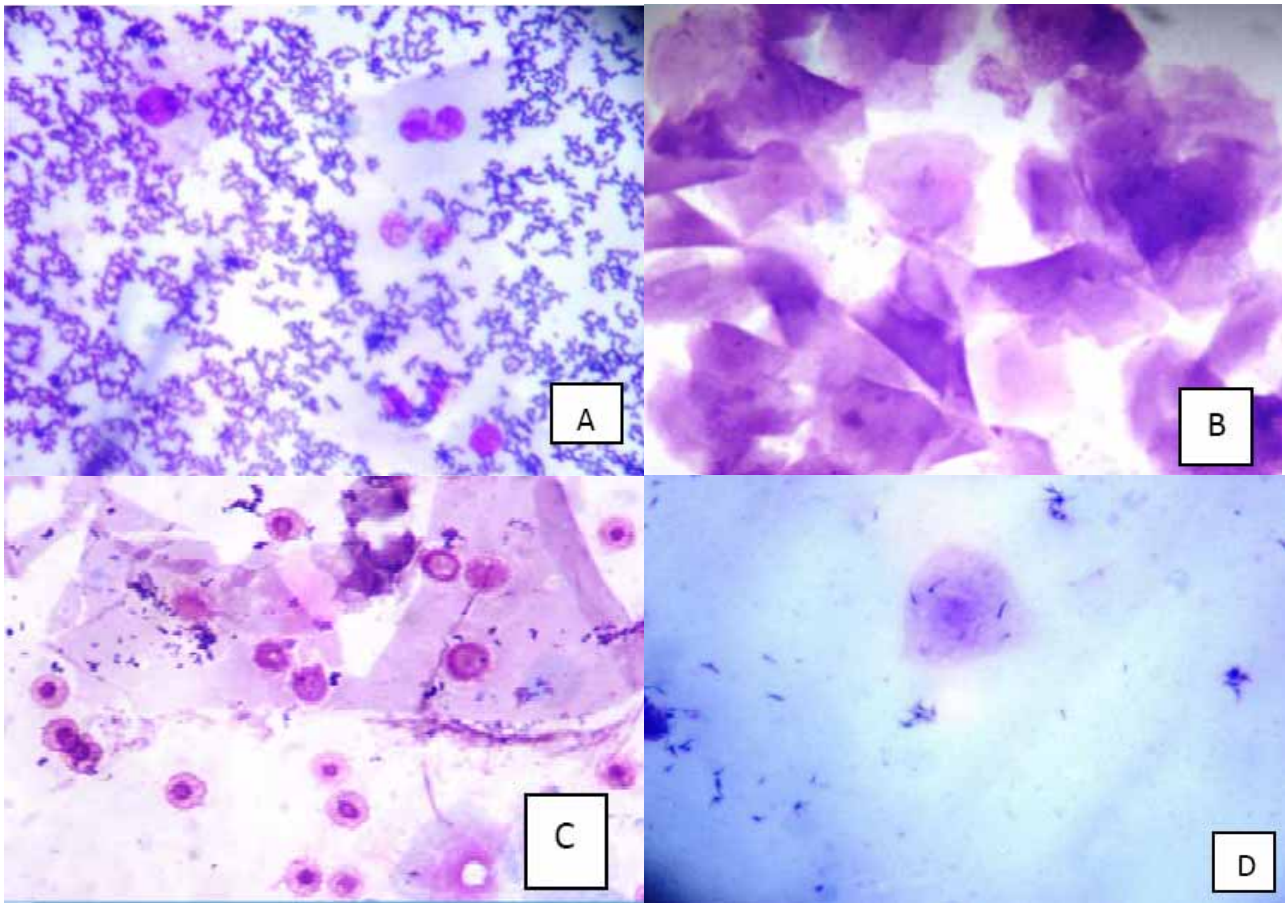

Figure 1: Exfolaitive vaginal cytology during different phases of estrous cycle in bitches. A. Proestrus (RBC and Parabasal cell). B. Estrus (Superficial cells). C. Diestrus (Intermediate cells and Neutrophils). D. Anestrus (Parabasal cell).

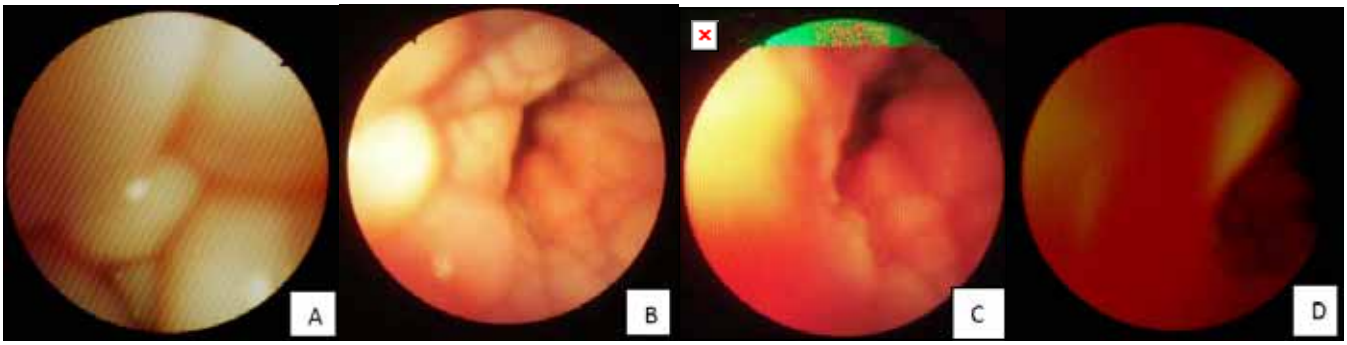

Figure 2. Vaginoscopic images during different phases of estrous cycle in bitches. A. Proestrus (edematous and round vaginal folds). B. Estrus (crenulation). C. Diestrus (banded hyperemic angulations). D. Anestrus (no angulations).

was clear (10). During anestrus, the background of smear was dirty which is as per the previous reports (5).

Endoscopy examination of the vaginal mucosa of bitches of CABG group appeared pink in colour and congested but without any mucosal shrinkage during proestrus. During this period, the edematous folds were found to be having rounded profiles. This might be due to thickening of mucosal epithelium and edema within sub mucosa due to the effect of increased estradiol levels which in turn led to retention of water in the vaginal mucosa

during proestrus. The onset of shrinkage of vaginal mucosa without angulations correlated with the onset of estrus which might be due to decreased levels of estradiol and consequent withdrawal of water from the vaginal mucosa. Dense creamy or paper white mucosa with angulated shrinkage during estrus was noticed which become increasingly sharp in profile. This indicated the fertile period in bitches. Cessation of mucosal shrinkage during diestrus and flat vaginal mucosa during anestrus might be due to low levels of estradiol (Figure 2). Similar studies were also carried by others (4,8-10). The plasma progesterone level was 0.37 ± 0.07 ng/ml first day of proestrus and it was recorded as 1.55 ± 0.19 ng/ml on day 8.00 ± 0.71 of proestrus in eCG treated bitches. Similar trend in progesterone profile during proestrus was noticed (2,8). The increased progesterone levels prior to estrus indicate the luteinization of preovulatory follicles which is a characteristic physiological feature in bitches (11-13) and also supported by histological evidence of luteinisation in the developing follicles prior to LH surge (15).

High per cent of superficial cells were appeared on day 10.67 ± 1.84 from first day of proestrus. The per cent of bitches conceived were 66.66 which were bred based on vaginal cytology. The shrinkage of vaginal mucosa without angulation was appeared on day 09.00 ± 1.58 and maximum shrinkage with angulations (crenulations) noticed on day 10.80 ± 2.08 after the onset of proestural bleeding in Cabergoline treated bitches. The per cent of conception was 83.33 in Cabergoline treated bitches. The increase in progesterone profiles noticed on day 08.00 ± 0.71 after the onset of proestrual bleeding in eCG. The per cent of bitches conceived were 83.33.

The litter size was 4.83 ± 0.83, 03.50 ± 1.12 and 04.33 ± 0.99 in Control, CABG and eCG group of bitches, respectively which is in line with other studies (14,16).

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References

1. Concannon PW, England G, Verstegen J, Linde-Forsberg C. Recent Advances in Small Animal Reproduction. International Veterinary Information Service New York. 2002.1-11p.
2. Johnston SD, Root Kustritz MV, Olson PNS. Canine and Feline Theriogenology. W B Saunder Company New York. 2001.16-41p.
3. Hewitt D, England GCW. Assessment of optimal mating time in the bitch. Small Anim Pract.2000:24-31.
4. Arun M. Semi and Non invasive techniques for detection of the stages of estrus cycle and pregnancy in bitches. [master's thesis]. India, ANG Ranga Agricultural University. 2001.46-75p.
5. Feldman EC, Nelson RW. Canine and Feline Endocrinology and Reproduction. 3rd ed. W.B. Saunders Philadelphia. 2004.486–538p.
6. Lindsay FEF, Concannon PW. Normal canine vaginoscopy. In Small Animal Reproduction and Infertility Ed. T.Burke Lea and Fibiger, Philadelphia. 1986.112-120p.
7. Lindsay FEF, Jeffcoate IA. Clinical methods of estimating the optimum period for natural and artificial insemination in the bitch. J Reprod Ferti.1993;47:556.
8. Sridevi P. Determination of ovulation and fertile breeding time to optimize conception in bitches. [dissertation]. India, Tamil Nadu Veterinary and Animal Science University. 2001.62-89p.
9. Patil MD. Induction of estrus in bitches using dopamine agonist Cabergoline. [master's thesis]. Tamil Nadu Veterinary and Animal sciences University Chennai. 2006.26–56p.
10. England GCW. Manual of small animal endocrinology. 2nd ed. Bra Small Anim Vet Assoc. 1998.43-46p.
11. Kustritz MR. Clinical canine and feline reproduction evidence based answers 2121 state avenue, Ames, Iowa 50014-8300, USA. 2010.97-98p.
12. Renton JP, Boyd JS, Harvey MJA, Ferguson JMF, Nickson DA, Eckersall PD. Comparison of endocrine changes and ultrasound as means of identifying ovulation in the bitch;. Res Vet Sci. 1992;53:74-79.
13. Rota A, Veronesi MC, Volpe S, Riccardi A and Battocchio M. Estradiol-17B, Progesterone and Testosterone Plasma Concentrations during Estrus in the Bitch. Vet Resea Commun. 2007;31(Suppl. 1):197-199.
14. Rota A, Mollo A, Marinelli L, Gabai G, Vincenti L. Evaluation of Cabergoline and Buserelin efficacy for oestrous induction in the bitch. Reprod Domes Anim. 2003;38:440-443.
15. Phemister RD, Holst PA, Spano JS, Hopwood M.L. Time of ovulation in the Beagle bitch. Bio. Reprod.1973;8:74–82.
16. Gobello C, Castex G, Dela Sota L, Corrada Y. Shortening of inter-estrus intervals with Cabergoline in bitches a clinical trial. J Amer Anim Hospi Assoc.2004; 40:115-119.