Short Communication

EXFOLIATIVE VAGINAL CYTOLOGY, VAGINAL pH AND VAGINAL ELECTRICAL RESISTANCE IN SPONTANEOUS AND INDUCED ESTRUS: A COMPARATIVE STUDY TO PREDICT FERTILITY IN BITCHES*

B.Jayasudha[1], K.G.Solmon Raju[2], K.C.S Reddy[3] and P.Nagaraj[4]

Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Rajendranagar. P.V.Narasimha Rao Telangana Veterinary University, Hyderabad – 500008.

Received: 13-03-2020 Accepted: 05-05-2020

ABSTRACT

A total of 18 anestrus bitches were randomly divided into 3 groups (G-I, G-II and G-III) and 6 cyclic bitches were considered as G-IV. Estrus was induced in G-I and G-II groups with Cabergoline @ 5 µg per kg body weight orally till the second day of proestrual bleeding and eCG + hCG @ 20 IU/Kg b.wt for 6 days with single injection of hCG @ 500 IU per bitch on day 7, respectively. G-III (anestrus control) bitches were treated with normal saline for 7days and G IV (estrus control) bitches were allowed to exhibit estrus spontaneously. In the present study, vaginal exfoliative cytology along with VER at estrus was recommended to predict best fertility among induced with cabergoline and cyclical estrus exhibited bitches.

Keywords: Cabergoline, hCG & eCG, Vaginal cytology, Vaginal Mucus Electrical Resistance,

INTRODUCTION

Bitches are non-seasonal monoestrous because they have only one oestrus cycle during each breeding season. There are several investigative methods for identifying the optimal mating time in bitches including measurement of vaginal mucus electrical resistance (VER), examination of exfoliated vaginal cells, progesterone level and vaginal pH. Vaginal cytology is a simple technique that can be used by practitioners to help characterize stages of the reproductive cycle of the bitch (Johnston et al., 2001). Exfoliative vaginal cytology alone was not sufficient in determining the optimal time period for mating among some bitches (Hiemstra et al., 2001). Hence, an attempt was made to compare the efficacy of vaginal cytology, vaginal pH and VER to predict précise ovulation period in bitches.

MATERIALS AND METHODS

A total of 18 clinically healthy bitches with history of secondary anestrus and 6 cyclical healthy bitches aged between 1.5 to 8 years (12 German shepherd and 12 Labrador) presented to the Veterinary Clinic Campus, College of Veterinary Science, Rajendranagar and Private Kennel located at Secunderabad were utilized for the present study. Anestrus was identified by history of prolonged quiescence of estrus behavior which lasted for 3-5 months (Noakes et al., 2001) and anestrus stage was also confirmed by typical findings on vaginal cytology and VER for the selection of bitches.

A total of 18 secondary anestrus confirmed bitches by Vaginal cytology, Vaginal pH and VER were selected and randomly divided in three groups i.e. Group-I, Group-II and Group-III likewise a total of 6 normal estrus exhibited bitches were selected i.e. Group IV. The groups were here after referred as G-I, G-II, G-III and G-IV.

Group I (G-I): A total of six bitches were induced estrus with cabergoline* @5µg per kg body weight orally till the second day of proestral bleeding. Group II (G-II): A total of six bitches were treated I/m with eCG** @20 IU/Kg b.wt for 6 days with single injection of hCG*** @ 500 IU per bitch on day 7. Group III (G-III): A total of six anestrus bitches were treated with normal saline for 7days. Group IV (G-IV): A total of six cyclic bitches were also treated with normal saline for 7 days were served as control Group.

The ovulation was assessed based on the parameters like percent cornified cells, vaginal pH, VER as mentioned in the Table-1 detailed below: At the time of ovulation, the per cent cornified cells in G-I, II, III and IV bitches were 85.17 ± 1.14, 81.41 ± 1.54, 0.00 ± 0.00 and 86.17 ± 1.14, respectively. Significantly ($p<0.05$) higher per cent of cornified cells were seen in G-IV. There were no cornified cells seen in G-III.

At the time of ovulation, the vaginal pH in bitches was noticed as 6.42 ± 0.06, 6.58 ± 0.05, 7.82 ± 0.03 and 6.35 ± 0.07 in G-I, II, III and IV bitches, respectively. Significantly ($p<0.05$) lower pH was recorded in G-IV bitches followed by G-I,G-II bitches at the time of ovulation. The vaginal pH was significantly ($p<0.05$) higher in G-III bitches.

During ovulation, the VER was recorded as 483.33 ± 77.35, 121.66 ± 2.07, 96.66 ± 3.33 and 566.66 ± 28.94 in G-I, II, III and IV bitches, respectively same

*Part of thesis submitted for partial fulfillment of MVSc thesis. 2&3 Professor, 4.Professor of Veterinary Medicine. *Email address: sreekrisla@gmail.com*

trend of VER of estrus was noticed at the time of ovulation in G-I and G-IV and lower VER was noticed in G-II and G-III group of bitches.

EXFOLIATIVE VAGINAL CYTOLOGY

The vulva was cleaned and vulval lips were separated by two fingers. A sterile swab was inserted inside to collect a sample of vaginal cells (Johnston et al.,2001). The swab was introduced at the dorsal commissure to avoid the clitoral fossa. The swab was then removed and rolled on two clean glass slides and then smears were prepared. One slide of vaginal smear was air dried and stained using field stain; second slide of vaginal smear was fixed and stained using Giemsa staining. Vaginal smears were collected on alternate days from the day second of proestrus till the day of diestrus. The per cent of parabasal cell, intermediate cells and cornified cells during different phases of oestrus cycle was estimated in spontaneous and induced groups.

VAGINAL pH

Determination of vaginal pH was done by inserting the indicator strips (Hi Indicator pH Paper, HIMEDIA) into vagina where it was touched to the vaginal wall for at least 3 sec. Evaluation of different stages of oestrus cycle was done after comparing the changes in colour of the indicator. The vaginal mucus pH was tested every alternate day till the day of diestrus (Ross, 2005).

VAGINAL MUCUS ELECTRICAL RESISTANCE (VER).

The vaginal mucus conductivity was measured with Draminski Dog Ovulation Detector. The electronic detector consisted of a measuring probe, a digital display block where readings were taken, and the handle with the on/off switch. The vulval lips were separated apart and delicately inserted the probe so that its end reached the lower depression which ought to be reached at a length of 3/4 of the probe. When this depth was reached some resistance was felt. The machine was carefully rotated the entire unit through 360 degrees (a full rotation) keeping the probe in the same position every time without moving the probe by either withdrawing or inserting it further so that the electrodes came into full contact with the vaginal mucus.

The vaginal mucus conductivity was measured from 2nd day of proestrus bleeding until the occurrence of the diestrus correlated by the appearance of parabasal cells in vaginal cytology

RESULTS AND DISCUSSION

The per cent cornified cells, vaginal pH and VER during different phases of oestrus cycle is presented in the table No.1.

During estrus, the range of cornified cells were 63.00±8.32 to 70.66 ± 1.72 against 0.00 per cent in anestrus bitches. The vaginal pH was around neutral in estrus but highly alkaline in anestrus bitches (7.86 ± 0.02). Similarly, the VER was higher in cabergoline induced estrus and spontaneously estrus exhibited bitches. Significantly ($p<0.05$) higher percent of cornified cells, lower pH and higher VER were recorded during estrus than the anestrus bitches. However regarding VER, there were variations within the induced estrus and spontaneously exhibited estrus. This might be due to the change in degree of hydration of vaginal tissue consequent to variation in endocrine profiles.

The time and intensity of maximum cornification varies among bitches and preclude its use to prospectively predict the exact time of receptivity to mating, the LH surge, or ovulation. (Johnston et al., 2001). The optimum time for natural breeding or artificial insemination is the estrus stage, when per cent of superficial and squamous cells in vaginal smears is above 80 per cent (Johnston et al., 2001 and Srinivas et al., 2004).

Significantly ($p<0.05$) lower pH (acidic) was recorded at the time of ovulation. Similar studies on vaginal pH were also recorded by (Labib et al., 2018) in bitches who attributed it to a progressive decrease in estrogen and gradual increase of progesterone levels. This peculiarity could be regarded as species specific for the bitch (Antonov et al., 2014).

Vaginal mucus impedance (VMI) is primarily in?uenced by progesterone concentrations but is also affected by the ratio of estradiol to progesterone concentration especially estradiol concentration (Gunzel et al., 1986), Decreased estradiol concentration have been reported to occur near the LH peak by Wright (1991) and Concannon et al., (1989) study, which found no signi?cant relationship between LH and VMI, suggest that vaginal impedance was not controlled by estradiol concentration. It may be that vaginal impedance was controlled primarily by progesterone concentration or, as it is in sheep, by the progesterone-to-estradiol concentration ratio. The unique pattern of changing progesterone concentrations in bitches (a result of pre-ovulatory luteinization of follicles) could be responsible for the unique pattern of changes in VMI, compared with other species. (Bartlewski et al.,1999). In nut shell vaginal parameters depend on progesterone concentration especially the changes of vaginal electrical resistance (Antonov et al., 2016).

However, it was noticed that there was no significant change of VER in eCG +hCG and anestrus control bitches when compared to other two groups. The variations in VER could be attributed to parity (Rezac et al., 2002), pre ovulatory LH profile, estrogen and progesterone levels at different stages of oestrus cycle

in mammals (Rezac, 2008). Apart from this variation could be attributed to depth of ovulation detector positioning (Antonov et al., 2014 and Rezac, 2008) and vaginal pH (Georgiev et al., 2012) in bitches.

The Vaginal mucus except epithelial cells and substances necessary for the survival of spermatozoa usually contains electrolytes (salts of sodium, magnesium, calcium and other cations) which determine its electrochemical reaction. The presence of electrolytes leads to ionization of estrus mucus and affects its electrical conductivity (Ahmed M et al., 2017). Based on these characteristics of estrus mucus, methods for determining the electrical resistance of vaginal mucosa has been developed as a method for detecting the optimum time for natural service or insemination (Rorrie et al., 2002). Hence, The VER was higher during ovulation both in cyclic (G-IV) and induced estrus (G-I).

It is concluded that at the time of ovulation the per cent cornified cells were more than 80 per cent with acidic vaginal pH and higher VER in bitches. Hence a combination of these methods could be practiced to predict fertility period in bitches to get optimum litter size.

ACKNOWLEDGEMENT

The authors are thankful to the staff of VCC, C.V.Sc, Rajendranagar for utilizing the clinical cases for study and Director, Dept of Veterinary & Animal Husbandry, Telangana State for providing the opportunity to pursue higher studies.

REFERENCES

Ahmed M, Chowdhury MK, Rahman MM, Bhattacharjee J and Bhuiyan MMU (2017). Relationships of electrical resistance of vaginal mucus during estrus with post-AI pregnancy in cows. *Bangladesh Journal of Veterinary Medicine* **15**(2):113-117.

Antonov A. L., J. Dineva and P. Geotgiev (2014) Dynamics of vaginal pH in the bitch during proestrus and oestrus. *Animal and Veterinary Sciences*. **2**(4): 101-104.

Antonov, A.L., J. Dineva and P. Geotgiev (2016) Influence of ovarian activity on vaginal Electrical resistance in the bitch during proestrus and estrus. Journal of Mountain Agriculture on the Balkans, **17**(5): 1070-1086.

Bartlewski PM, Beard AP, Rawlings NC. (1999) Therelationship between vaginal impedance and serum concentrations of estradiol and progesterone throughout the sheep estrous cycle. *Theriogenology,* **51**(4):813–827.

Concannon, P. W., McCann, J. P., & Temple, M. (1989). Biology and endocrinology of ovulation, pregnancy and parturition in the dog. *Journal of reproduction and fertility. Supplement **39***, 3–25.

Georgiev.P,A.Antonov, R. Grancharova, and J. Dineva (2012) "Dynamics of vaginal electrical resistance in the bitch during proestrus and estrus", Second Scientific Students Conference, Abstract book, p. 25.

Gunzel, A.R., Koivisto, P., Fougner, J.A., (1986). Electrical resistance of vaginal secretion in the bitch. Theriogenology **25**, 559–570.Hiemstra, M., A. C. Schaefers-Okkens, E. Teske and H. S. Kooistra (2001) The reliability of vaginal cytology in determining the optimal mating time in the bitch. *Tijdschrift Voor Diergeneeskunde*. **126** (21): 685-689.

Johnston, S.D., M. V Root-Kustritz and P. N. Olson (2001) "In Canine and Feline Theriogenology". W.B.Saunders, Philadelphia. Pp.16-66.

Faten M Labib., Hassan H. Mansour, Atef B. Mahdy, Asmaa A. Abdallah (2018) Predicting the optimal time of breeding and the possible approaches for treatment of some estrus cycle abnormalities in bitches. *Slov Vet Res* **55**(20): 125–135.

Noakes DE, Parkinson TJ, England GCW, Arthur GH; (2001) Arthur's Veterinary Reproduction and obstetrics. 8th edition, Harcourt Publishers Limited, Pennsylvania.

Rezac P (2008), Potential applications of electrical impedance techniques in female mammalian reproduction. *Theriogenology,* **70**(1), 1–14.

Rezac P, Kukla R, Poschl M (2002): Effect of sow parity on vaginal electrical impedance. *Animal Reproduction Science* **72**(3-4), 223–234.

Ross, A. (2005) "Investigations in the female dogs vaginal aerobic flora and pH", Dissertation, Berlin.

Srinivas, M., N. Lakshmi Rani, K. Suresh & M. Sreenu, (2004) Vaginal exfoliative cytology as a tool in diagnosing reproductive disorders in bitches. *Intas Polivet*, **5**(2), 354–356.

Wright, P. J. (1991) Practical aspects of estimation of the time of ovulation and insemination in the bitch. *Aust. Vet. J.* **68**(1): 10-13.

Table No. 1 : Mean per cent cornified cells, vaginal pH and VER during different phases of oestrus cycle

Stage of Oestrus cycle	Group	Cornified cell (per cent)	Vaginal Ph	VER (ohms)
Proestrus	G-I	4.47 ± 1.42^{ab}	7.80 ± 0.08	120.29 ± 13.92^{a}
	G-II	6.83 ± 2.64^{b}	7.73 ± 0.08	113.88 ± 4.28^{a}
	G-III	0.00 ± 0.00^{a}	7.88 ± 0.05	91.05 ± 2.63^{a}
	G-IV	4.68 ± 2.43^{ab}	7.74 ± 0.06	195.62 ± 26.14^{b}
Estrus	G-I	63.50 ± 6.04^{b}	7.30 ± 0.10^{a}	286.66 ± 35.93^{b}
	G-II	70.66 ± 1.72^{b}	7.13 ± 0.02^{a}	125.00 ± 2.23^{a}
	G-III	0.00 ± 0.00^{a}	7.86 ± 0.02^{b}	106.66 ± 2.10^{a}
	G-IV	63.00 ± 8.32^{b}	7.02 ± 0.14^{a}	408.57 ± 55.05^{c}
Ovulation	G-I	85.16 ± 1.13^{bc}	6.41 ± 0.06^{ab}	483.33 ± 77.35^{b}
	G-II	81.41 ± 1.54^{b}	6.58 ± 0.05^{b}	121.66 ± 2.07^{a}
	G-III	0.00 ± 0.00^{a}	7.81 ± 0.03^{c}	96.66 ± 3.33^{a}
	G-IV	86.16 ± 1.13^{c}	6.35 ± 0.07^{a}	566.66 ± 28.94^{b}
Diestrus	G-I	30.91 ± 3.11^{b}	7.47 ± 0.13^{a}	166.66 ± 25.56^{ab}
	G-II	29.83 ± 1.92^{b}	7.75 ± 0.07^{ab}	90.00 ± 4.47^{a}
	G-III	0.00 ± 0.00^{a}	8.11 ± 0.06^{b}	90.00 ± 2.58^{a}
	G-IV	34.91 ± 2.98^{b}	7.74 ± 0.13^{ab}	212.50 ± 26.43^{b}

Figure bearing different superscripts column wise differed significantly ($p<0.05$)