Efficacy of Exfoliative Vaginal Cytology and Progesterone Assay on Fertility in Bitches

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Abstarct: The present research work was conducted to study the efficiency of exfoliative vaginal cytology (EVC) and progesterone assay for detection of ovulation period as well as fertility in bitches. In Group-I, exfoliative vaginal cytology (EVC) was performed at alternate days in bitches (n=6) in pro-oestrus period and when cornification index reached more than 80%, suggested for mating. In Group-II, exfoliative vaginal cytology (EVC) was performed at alternate days in bitches (n=6) presentenced in pro-oestrus period and when cornification index reached more than 80%, serum progesterone level was estimated. Based on the progesterone levels, the bitches were suggested mating accordingly. The mean cornification index (%) was 89.33±2.37 and 88.17±2.12 in Group I and II, respectively and non-significant difference within the groups (P>0.05). The mean predicted day ovulation was 9.33±0.80 and 8.16±0.98, respectively in Group I and II and non-significant within the groups (P>0.05). The mean ± SE serum progesterone after 80 % cornification index was 3.79 ± 0.55 ng/ml. The mean ± SE of litter size were 5.83 ± 0.79 and 4.50 ± 0.67 ain Group I and II and showed non significant difference between the groups.

Keywords: Bitches, Ovulation, Vaginal cytology, Progesterone.

INTRODUCTION

The most common problem encountered in canine reproduction is the “potentially” infertile bitch. Majority of the infertile bitches actually become victims of mismanagement. The bitches show considerable variations in duration of proestrous, estrus and time of ovulation. When standard mating regimes are imposed in such bitches leads to fail to conceive and is erroneously considered to be infertile [1]. The majority of the bitches presented with history of infertility are in fact fertile [2]. The most common cause of conception failure with reported incidence between 40 and 50% is mistimed breeding time [3].

The determination of the time of ovulation is of major importance to the management of normal reproduction in the bitch. The determination of the time of ovulation indicates the period most appropriate for mating or insemination. Canine ovulation occurs approximately 44h after LH surge [1]. Moreover, the oocytes ovulated in canines are primary oocytes and undergo further development in the distal portion of the oviduct to form secondary oocytes before fertilization. Fertilization occurs 2 to 3 days after ovulation when the ova, which are ovulated, have completed meiotic divisions [4].

The ovulation date provides a more accurate basis for the estimation of the time of whelping than do mating dates, with whelping occurring around 63 days after ovulation [5]. Apart from failure of conception, determination of ovulation time also helps to carry out artificial insemination with fresh or chilled or frozen thawed semen, mating with sub-fertile studs and predicting the whelping date.

Many procedures for the estimation of the time of ovulation have been described. These include direct observation of the ovaries [6] and indirect methods based on the day of the cycle, behavior of the bitch, vaginal cytology, plasma lutenizing hormone (LH) or progesterone concentrations and the nature of the vulval discharge [4]. Vaginal cytology defines the stage of the oestrus cycle because the vaginal epithelium undergoes morphologic changes under estradiol and LH transition. It is based on determination of cyclic cellular changes occurring in the vaginal epithelium as a result of reproductive hormone levels, especially estrogens [7]. Due to the estrogenic influence, an increase in cell layers, keratinisation and exfoliation is observed in the follicular phase during proestrus, such that the 3-4 layered epitheliums in anoestrus becomes 20-layered during oestrus. The cells change characteristically in size and nuclear morphology. The

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popularity of this test is high due to the simplicity and limited equipment necessary for the test. Vaginal cytology offers a rapid, inexpensive, and reliable in-clinic method to evaluate stages of the oestrus cycle in the bitch.

The assessment of circulating progesterone concentrations is the most used approach for detecting the lutenizing hormone (LH) surge which is found to be concomitant with the initial marked increase of the circulating progesterone [8]. After the LH surge, the serum progesterone rises from around 1ng/ml, during anoestrus and early proestrus to 4-5ng/ml at ovulation. Radioimmunoassay (RIA) or enzyme immunoassay provides accurate and reliable results, but these have disadvantage of high expense and long turn- around time. In practice, a blood concentration of progesterone ≥5 ng/ml considered indicative of ovulation [9]. The specificity progesterone assay gives a more accurate estimation of each bitch individually increasing the likelihood of successful pregnancy [10]. Considering the importance of detection of ovulation time in canine breeding, this study aimed to detect precise ovulation time by vaginal cytology, progesterone assay and its effect on litter size in bitches.

MATERIAL AND METHODS

The present research work was carried out at the Department of Animal Reproduction, Gynaecology and Obstetrics and Teaching Veterinary Clinical Complex, Post Graduate Institute of Veterinary and Animal Sciences, Akola during the period from July 2018 to July 2019. A total 24 sexually matured clinically healthy Labrador retriever and German shepherd breed bitches in between 3 - 6 years of age were selected. A complete history like age of the bitches, vaccination and deworming status, any reproductive abnormalities, previous failure of conception, and last date of whelping, previous litter size were recorded. The vagina was explored with for gloved finger digit to rule out any vaginal abnormalities. The specific reproductive examination like onset of proestrual bleeding, vulval oedema, nature of vaginal discharge and colour of discharge, postural signs exhibited by bitches like flagging of tail, upward stretch of vulva and rigidity of the hind limbs were noted. The selected bitches were divided into two groups.

Group I

Bitches (n=6) presentenced in pro-oestrus period were selected and exfoliative vaginal cytology (EVC) was performed at alternate days for detection of cornification index (CI). When the cornification index reached more than 80%, the bitches were suggested for mating on alternate days.

Group II

Bitches (n=6) presentenced in pro-oestrus period were selected and exfoliative vaginal cytology (EVC) was performed at alternate days for detection of cornification index (CI). When cornification index reached more than 80%, serum progesterone level was estimated. Based on the progesterone levels, the bitches were suggested mating accordingly.

Vaginal smears were collected from mid-vagina and the vaginal mucosa was wiped with a combination of circular, twisting and back-and-forth motions. A smear was prepared on a clean glass side and stained using Giemsa stain. The slide was fixed using methanol fixative by dipping the slide in it for a few seconds. The slide was covered with diluted stain and kept still for 20 minutes. The slide was washed with distilled water and air dried. Once proestrus was confirmed, swabs were collected every alternate day until cornification index reached or surpassed 80%. The different types of cells like parabasal, intermediate, superficial and anuclear cells were observed under microscope. Total 100 cells from 4 fields were examined and differentiated. Average was taken according to number of slides prepared and cornification index was recorded based on the attained value of cells which was expressed in percentage (%).

About 2-4 ml blood was collected aseptically from the cephalic/ saphenous vein. Immediately after the collection, it was transferred into a clot activator vial. The vial was kept in a standing position to facilitate the clotting and separation of serum. The vial was then send to Pathology laboratory which is located Akola at room temperature immediately for progesterone estimation. The serum progesterone was measured using ST AIA Pack Progii ELISA progesterone kits. The data of this investigation was analyzed by employing suitable statistical design as recommended by Snedecor and Cochran (1994) [11].

RESULTS AND DISCUSSION

The mean cornification index (%) was 89.33±2.37 and 88.17±2.12 in group I and II, respectively. The results are non-significant within the groups. The mean predicted day ovulation was 9.33±0.80 and 8.16±0.98, respectively in Group I and II and non-significant within the groups (P>0.05). The mean ± SE serum

Table 1: The Mean (%) of Different Types of Vaginal Exfoliative Cells, Cornification Index, Predicted Day of Ovulation and Litter Size in Bitches

Groups	Cornification index (%)	Parabasal %	Intermediate %	Superficial %	Anuclear %	Predicted day of ovulation	Litter size
I (n=6)	89.33±2.37	3.66±1.19	7±1.1	51.83±7.0	37.5±8.02	9.33^{NS} ±0.80	5.83^{NS} ± 0.79
II (n=6)	88.17±2.12	4.67±1.36	7.17±1.49	47±7.45	41.16±5.85	8.16^{NS} ±0.98	4.50^{NS} ± 0.67

progesterone after 80 % cornification index was 3.79 ± 0.55 ng/ml in Group II. The percentages of different types of vaginal exfoliative cells within the four groups are non-significant. The mean ± SE of litter size were 5.83 ± 0.79 and 4.50 ± 0.67 ain Group I and II and showed non significant difference between the groups.

The result of present study for cornification index is in agreement with Raikar (2018) [12] who recorded 88.75 ± 2.05 % cornification index on the day of ovulation. Tehlan (2013) [13] reported 94.43 ± 3.90% and Bante *et al*., (2018) [10] observed cornification index of 96.87±0.43 during ovulation which is higher than the findings of present study. The values reported by Hahn *et al.,* (2017) [14] who reported cornification index of 80% which is lower than the result of present work. Linde and Karlsson (1984) [15] reported a cornification index within range of 80 to 90% which is in accordance with the present findings. The variation in cornification index may be due to days of oestrus [16], level of estrogen, progesterone concentration [14], method of detection [10], thickness of vaginal epithelium, method of collection of swab and individual variation among the breed.

The finding of day of ovulation recorded in the present pursuit is in harmony with Phemister *et al.* (1973) [17] recorded mean 10 days required for ovulation after onset of bleeding. The longer days required for ovulation were reported as days 16 [6]; 11 days in spontaneous oestrus [18]; 12±1.63 days [13]; 13.92±0.82 days [12]; 12 days [19] and 12.70±1.19 days [10]. The lower days required for ovulation were reported as 8.2 day after onset of proestrus in GnRH treated bitches [18] as well as 6.9±1.6 days after 80 % cornification index [20]. Ovulation is considered to have occurred 6.9 ± 1.6 days after >80% cornification index was reported by Bouchard *et al.* (2018) [20]. Based on this hypothesis, in the current research findings ovulation was occurred 9.58 ± 0.53 day in bitches in all groups.

The result of present findings for progesterone level is in concurrence with Hase *et al.* (1999) [21] who recorded 2.34 ng/ml two days after LH peak. The higher progesterone level than present study findings were observed by Phemister *et al.* (1973) [17] reported 5.7ng/ml while Linde and Karlsson (1984) [15] reported 5.44 ng/ml and Bouchard *et al.* (2018) [20] reported 4.9 ng/ml plasma progesterone at ovulation. Wildt *et al.* (1979) [6] reported 7.1 ng/ml; Tehlan (2013) [13] reported 9.52 ± 4.97 ng/ml plasma; Raikar (2018) [12] reported 10.75 ng/ml and Bante *et al.* (2018) [10] observed 8.67 ng/ml progesterone level at ovulation which much higher than the present findings. The variation in days of ovulation may be due to induced or spontaneous oestrus [18], level of progesterone [19], presence of fertile male and individual variation among breed.

The variation in the level of progesterone is comparatively lower than reported by different researcher because the progesterone was recorded after 80 % cornification index in the present study while at ovulation by other scientist. This period is earlier approximately 24-48 hrs. before LH peak and canine ovulation occurs approximately 44h after Lh surge [1]. The variation also may due to number of luteinized follicles and individual variation among the breed.

CONCLUSION

The vaginal exfoliative cytology is easiest, time saving and economical technique for detection of ovulation compared to progesterone assay without affecting litter size in bitches.

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