COMPARATIVE STUDY OF DETERMINATION OF OVULATION TIMING IN BITCHES

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The present study was undertaken for Determination of ovulation timing in bitches by using different techniques in bitches. Progesterone estimation was used to predict LH surge and ovulation. Progesterone level at LH surge and at ovulation was 3.17 ± 0.95ng/ml and 8.67±3.56ng/ml, respectively. Progesterone level was at peak during ovulation. The average of electronic ovulation detector reading before LH surge was 347.91 ± 83.71kΩ, at LH surge 455 ± 105.74kΩ and at ovulation 475.41 ± 88.61kΩ. pH was estimated and compared with progesterone levels at different stages of oestrous cycle. There were significant differences of different stages of oestrous for cornification Index percentage, P4 and Electronic Ovulation Detector and pH during ovulation. Thus, it could be concluded that Leishman's stain can be used preferably best for vaginal cytology to determine ovulation timing in bitches.

Keywords: Ovulation timing, Serum progesterone, Vaginal cytology.

The bitches are monoestrus, they ovulates only once or twice a year, at 5-12 months interval, they have prolonged estrus period and eggs are ovulated as primary oocytes, which only undergo maturation after one or more days in the oviduct. Further, canine spermatozoa have a prolonged survival time in the genital tract of the bitch (Concannon *et al*., 1983).

Vaginal cytology cannot be used alone to detect ovulation prospectively as it is not repeatable and precise enough. At the end of the heat period, the "onset of vaginal metoestrus", when there is a sudden increase in intermediate cells and parabasal cells, occurs around 5 days after ovulation (Nothling and Volkmann, 2003). But, it only helps to detect ovulation retrospectively. Wright (1990) found that the precision of the determination of the time of ovulation was 12 days based on vaginal smears (reaching an eosinophilic index of 100%) and 6 days based on the first metoestrus smear.

Few investigators have studied, electrical resistance of the fluids of the anterior vagina by means of electronic probes (Edward and Levin, 1974; Gertland *et al*., 1976) Vaginal cytology is a simple technique that can be used by practitioners to characterize stages of the reproductive cycle of the bitch. The specificity of LH and progesterone assay gives a more accurate estimation for each bitch individually increasing the likelihood of a successful pregnancy. Electrical resistance of vaginal mucus is relatively new and a nontraditional approach at the diagnostics of various reproductive disorders in mammals is the measurement of the electrical resistance of the vagina (impedance). Hence the present study is aimed to suggest breeding time based on vaginal cytology, electronic ovulation detector, pH and progesterone profile.

Materials and Methods

The present study was conducted in 24 bitches of different breeds and age presented to Teaching Veterinary Clinical Complex, Nagpur Veterinary College, Nagpur. These bitches were diagnosed to be in pro-estrus, oestrous based on history, behavioral signs, physical examination and vaginal electrical resistance measurement.

The samples were collected using the cotton swab technique. Total 24 vaginal smears were prepared. The prepared smear was air dried and smeared with Leishmain's stain for 15-20 minutes. Total 100 cells were counted under microscope at 40X and the cytoplasm, nucleus and background and quality of stains were compared to determine

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ovulation timing. During pro-estrus, parabasal cells and intermediate cells RBC's and other cells like neutrophils are observed while during oestrous anuclear superficial cells and superficial cells. The bitches were examined for vaginal cornification starting from proestrus and subsequently every other day until a vaginal cornification index of a minimum of 90 % was recorded.

Along with this vaginal mucus electrical resistance measurements were also taken with electronic ovulation detector daily. The vulva was cleaned with a dry paper towel and the probe was inserted into the vagina. It was followed by a three times pressing the button and full rotation (360) so that the electrodes come into full contact with the vaginal mucus.

Vaginal pH was also measured by using strips 2 to 10.5. The cotton swab was inserted inside the vagina and rolled over the pH strip and different stages were determined after comparing the change in colour of the indictor.

The blood samples were also obtained for the estimation of serum P4 concentration. The serum P4 concentration was determined by using an ELISA kit. The serum P4 concentrations in these animals were assayed again to identify the initial sharp rise in preovulatory serum P4 concentrations. The initial sharp rise in serum P4 concentrations was defined as that concentration which was at least 1 ng /ml higher than the concentrations recorded during the immediate previous assay. Once such a sharp rise in serum P4 was recorded, the owners were advised to get their bitches mated twice, once on the day when the initial rapid rise in preovulatory serum P4 concentration was identified and again 48 hrs later.

Result and Discussions

In the present study was ovulating timing was determined by comparing values of vaginal electrical resistance, the level of serum progesterone along with vaginal cytology and Vaginal pH during pro-estrus, oestrous, LH surge and at ovulation in the bitches.

The average day of proestrus 6.54 ± 1.06 days ranged from 4 to 9 days was oestrous 8.74 ± 0.19 days ranging from 8 to 10 days, LH surge was 10.52 ± 0.20 ranging from 10 to 14 days, and ovulation 12.70±1.19 days ranging from 11 to 16 days.

The average cornification index during oestrous, at LH surge predicted by progesterone estimation and at ovulation was 85.62 ± 1.11% ranging from 74.5 to 95.5% , 93.89 ± 0.57% ranging from 86.5 to 97.5%, while 96.68 ± 0.35% ranging from 93.5 %to 98.5% respectively,

The average progesterone level during pro-oestrus was 0.431 ± 0.05 ng/ml ranging from 0.4 to 0.98 ng/ml, at oestrous 1.42 ±0.12 ng/ml ranging from 0.7 to 2.56 ng/ml ,at LH surge 3.17±0.19 ng/ml ranging from 1.52 ng/ml - 4.87ng/ml and during ovulation 8.677±0.72 ng/ml ranging from 5.56 to 12.05 ng/ml.

In 15 (65%) bitches a transient peak at the middle of proestrus was observed, followed by a mild decrease on the next day and after that the values started to increase again. In the remaining 10 (35%) animals a gradual increase of impedance during proestrus was observed.

There are two peaks of the values of vaginal impedance ("false peak" at the second half of proestrus and second on the day of ovulation), which we also found, although the first of them was registered at different times during pro-estrus in 75% of animals.

The peak values of vaginal resistance during estrus were observed between 9th and 13th day after the onset of proestrus. Some individual variations were observed. In 20 (86.5%) of the experimental animals was recorded statistically significant peak of the impedance between 290 to 720 and the average was 455±105.74, coinciding with the day of ovulation, followed by a sharp decline on the next day , and the trend to decrease was maintained until the end of estrus.

In the remaining 3 (12.5%) of animals the difference between the occurrence of the peak values of vaginal impedance and the ovulation was within a day. The average values of all the experimental animals on the 1st day of ovulation were 475.41±88.61

ranging from 350 to 610. The present results show some specifics in the bitch which could not be found in the previous studies e.g. of Gunzel *et al.* (1986).

During pro-oestrus vaginal electrical resistance values varied between 80 and 450Ω and the average was 188.75 ± 17.08 Ω and as stage progresses the values were gradually increased and were from 347.91± 17.08 Ω ranging from220 to 470 Ω. While during LH surge the average was 455 ± 21.58 Ω ranging from 290 to 720 Ω and during ovulation average was 475.41 ± 18.08 Ω ranging from 350 to 610 Ω.

The mean vaginal pH values in the bitches at the first day of pro-oestrus were between 8.02 ± 0.09 ranging from 7.2 to 8.8. This parameter decreased with the progress of the proestrus and the entering into the estrus. During oestrous the average pH value was 7.42 ± 0.05 ranging from 7 to 7.9 while during LH surge 7.07 ± 0.03 ranging from 6.7 to 7.2 at ovulation 6.59 ± 0.05 ranging from 6.2 to 6.9. Individually at the time of ovulation vaginal pH ranged between 6.2 and 6.9. In the days following ovulation a gradual increase in mean values was detected. It was found that the vaginal pH values in the bitch are lowest at the time of ovulation, with individual variations and exceptions.

It can be concluded that there was significant differences at different stages i.e. during (proestrus, oestrous, LH surge and ovulation) for cornification Index % and P4 electronic ovulation detector reading and pH. It observed that at ovulation stage all the parameters CI, P4, DR and pH are much more effective than any other stage.

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