

EFFICACY OF HUMAN CHORIONIC GONADOTROPHIN (hCG) HORMONE ON LITTER SIZE IN BITCHES

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The present research work was conducted to study the efficiency hCG hormone on litter size 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 and 500 IU hCG i/v was administered to each bitch after first mating only. In Group-II, exfoliative vaginal cytology (EVC) was performed at alternate days in bitches (n=6) presented 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 and 500 IU hCG i/v was administered to each bitch after first mating only. The mean cornification index (%) was 80.38 ± 3.27 and 82.83 ± 5.99 in Group I and II, respectively and non-significant difference within the groups ($P > 0.05$). The mean predicted day ovulation was 10.50 ± 1.33 and 10.33 ± 0.98 , respectively in Group I and II and non-significant within the groups ($P > 0.05$). The mean \pm SE of litter size were 4.83 ± 0.94 and 5 ± 1.36 in Group I and II and showed non significant ($P > 0.05$) difference between the groups. From the study it has been concluded that hCG @ 500 IU i/v after first mating has no effect on litter size in bitches.

Keywords: Bitches, Cytology, hCG, Progesterone, Ovulation.

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 (Wright and Parry, 1989). 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 (Concannon, 2011). 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 (Ververdis *et al.*, 2002). The specificity progesterone assay gives a more accurate estimation of each bitch individually increasing the likelihood of successful pregnancy (Baarton and Wolf, 1988). 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. One of the methods of increase in liter size and pregnancy in bitches by administration of luteinizing hormone after mating or 24 hrs after breeding (Yathish Kumar *et al.*, 2013).

Materials 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 12 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 proestral 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.

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 slide 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 serum progesterone was measured using ST AIA Pack Progii ELISA progesterone kits.

Group – I: Bitches (n=6) presented 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 and 500 IU hCG i/v was administered to each bitch after first mating only.

Group – II: Bitches (n=6) presented 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 and 500 IU hCG i/v was administered to each bitch after first mating only. The data of this investigation was analyzed by employing suitable statistical design as recommended by Snedecor and Cochran.

Results and Discussion

The mean cornification index (%) was 80.38 ± 3.27 and 82.83 ± 5.99 in group I and II, respectively. The results are non-significant within the groups ($P > 0.05$). The mean predicted day ovulation was 10.50 ± 1.33 and 10.33 ± 0.98 , respectively in Group I and II and non-significant within the groups ($P > 0.05$). The mean \pm SE serum progesterone after 80 % cornification index was 2.85 ± 0.29 ng/ml in Group II. The percentage of different types of vaginal exfoliative cells within the four groups are non-significant ($P > 0.05$). The mean \pm SE of litter size were 4.83 ± 0.94 and 5.00 ± 1.36 in Group I and II and showed non significant ($P > 0.05$) difference between the groups.

Table-1. THE MEAN (%) OF CORNIFICATION INDEX, PREDICTED DAY OF OVULATION AND LITTER SIZE IN BITCHES

Groups	Cornification index (%)	Predicted day of ovulation	Litter size
I (n=6)	80.38 ± 3.27	10.50 ± 1.33 ^{NS}	4.83 ± 0.94 ^{NS}
II (n=6)	82.83 ± 5.99	10.33 ± 0.98 ^{NS}	5.00 ± 1.36 ^{NS}

The result of present study for cornification index is in agreement with Hahn *et al.*, 2017, who reported cornification index *Indian Journal of Canine Practice* ISSN: 2277-6729 e-ISSN: 2349-4174

of 80%. The finding of day of ovulation recorded in the present pursuit is in accordance to Phemister *et al.* 1973, who also *Volume 13 Issue 2, December, 2021* (<http://creativecommons.org/licenses/by-nc/4.0/>)

recorded mean 10 days required for ovulation after onset of bleeding. The result of present findings for progesterone level is in concurrence with Hase *et al.* 1999, who recorded 2.34 ng/ml two days after LH peak. This period is earlier approximately 24-48 hrs. before LH peak and canine ovulation occurs approximately 44h after Lh surge. The variation also may due to number of luteinized follicles and individual variation among the breed.

The result of present findings for effect administration after mating are similar to the report of Yathish Kumar *et al.* 2013, who reported higher litter sizes of 6.28 ± 0.47 treated with 500 IU hCG i/v and 6.57 ± 0.57 treated with 1000 IU hCG i/v after mating while 5.83 ± 0.70 in control group.

Conclusions

The vaginal exfoliative cytology is easiest, time saving and economical technique for detection of ovulation and hCG @ 500 IU i/v after first mating has no effect on litter size in bitches.

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