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The Clinical Follow-Up of Estradiol Benzoate Priming During Induction of Estrus With Cabergoline in Dogs

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Induction of estrus is used to improve reproductive efficiency of female dogs. In this study, 11 adult healthy female dogs were selected at anestrus stage. The dogs were assigned to treatment (6 dogs) and control groups (5 dogs). Single dose of estradiol benzoate was injected in treatment group at day 0 (0.01 mg/kg, IM). Dogs in both groups received cabergoline (5 μg/kg orally) from day 7 to the onset of proestrus. Vaginal cytology and blood samples were taken twice a week during study. Average time to the onset of proestrus was 10.33 ± 4.2 and 15 ± 7.5 days in the treatment and control groups, respectively ($P = .08$). The differences in time to the onset of estrus phase in the treatment group (14.67 ± 5.9 days) and control group (18.67 ± 10.8 days) were significant ($P < .05$). The average length of proestrus phase in treatment and control groups was 5.33 ± 2.2 and 8 ± 4.6 days, respectively and their differences were significant ($P < .05$). Average length of estrus phase in treated dogs with estradiol benzoate was 8.57 ± 3.5 days but it was 8 ± 4.6 days in control group ($P > .05$). Administration of cabergoline caused significant decrease of prolactin concentration in both groups ($P < .01$). The difference in serum prolactin concentration between treatment and control was not significant. The effect of cabergoline on serum prolactin concentration was not affected by administration of estradiol benzoate in treatment group ($P > .05$). As a result, administration of estradiol benzoate 1 week before cabergoline improved induction and synchronization of estrus in dogs.

Introduction

One of the problems facing dog breeding programs is monoestrus cycle. The average estrus cycle is 31 weeks[1] ranging from 16-56 weeks[1,2]; it means that the interval between 2 estruses of bitch is 5-7 months and in some, increase to 18 months. In addition, the time required for repairing of the uterus (pregnant or nonpregnant) is atleast 135 days.[3] Prolonged estrus cycle and variable distance between estruses are due to variability of anestrus time in different dog breeds. Because of the prolonged estrus cycle in female dogs, they have little opportunity to conceive during their reproductive life.[4,5]

Multiple programs have been developed to induce estrus in the bitches to establish fertile estrus within 4-5 month intervals. Several drugs, including eCG, hCG, FSH, LH, GnRH, estrogen, bromocriptin, and cabergoline have been used in various programs.[6–10] Each of drugs and programs has results, advantages and disadvantages of their own. For example, in cases of eCG and hCG therapy, although most of the dogs demonstrate good signs of estrus, some of follicles fail to ovulate.[11] Cabergoline-based protocols have been introduced as an appropriate estrus inducer, which is consistent with normal endocrine events and better fertility.[12]

Cabergoline is a dopamine agonist that reduces concentration of prolactin similar to late anestrus.[13] There are 3 major problems in the use of cabergoline: (1) the high price and long-term treatment (average duration of treatment with cabergoline is more than 2 weeks and the drug is relatively expensive).[14] (2) restrictions on drug use (according to studies conducted in recent years, the minimum effective dose of cabergolin to induce estrus in bitches is 5 μ/kg, but human drugs on the market are 0.5, 1, or 2 mg tablets),[11] and (3) the major side effects of dopamine agonist prescription are hair discoloration and vomiting,[15] but vomiting in patients taking cabergoline compared with bromocriptine occur less frequently.[16] No side effects such as nausea and vomiting were observed with the use of cabergoline for German Shepherd Dogs.[16,17]

The pituitary sensitivity to GnRH and ovarian responsiveness to LH and FSH increases before proestrus.[18,19] Serum FSH concentration is high in all anestrus periods (due to lack of negative feedback of estrogen and inhibin) and LH concentration is low except in late anestrus.[6] Approximately 30 days before the start of proestrus, a small but significant rise in serum estradiol occurs.[19] In this study estrdaiol priming before cabergoline administration may mimic this phenomenon.

The mRNA expression levels of α and β receptors increase in the hypothalamus, pituitary, and ovaries in late anestrus to proestrus in dogs.[20] These observations suggest that estradiol is an intermediate for beginning of hypothalamic-pituitary-ovarian axis activity by increasing the release of LH pulses.[21] So, estrogenic compounds are used in estrus induction programs to induce LH release and establish its receptors in follicles before ovulation. In anestrus dogs, 17-β estradiol treatment has increased GnRH concentration in the hypothalamus.[22] The purpose of the study was comparison of 2 protocols of estrus induction and synchronization with estradiol benzoate and cabergoline.

Materials and Methods

This study has been approved by the Iranian laboratory animal ethics framework under the supervision of the Iranian Society for the Prevention of Cruelty to Animals and Shiraz University Research Council. Oiled solution of 1 mg cabergoline tablet (Dostinex, PFIZER, Italy) was prepared in the following method, which each milliliter of solution containing 100 μg of cabergoline. Initially the tablet was powdered in a porcelain mortar. The resulting powder was dissolved in coconut oil as much as possible and the

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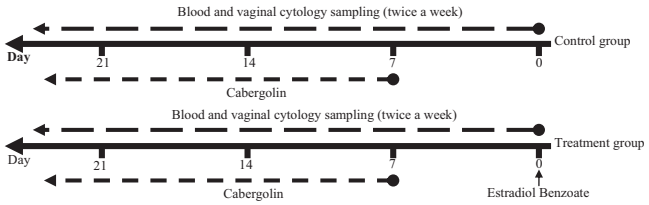

Fig. 1. Treatment and control groups with timing of medicine administration and sampling.

remaining powder resolved by adding water and propylene glycol to the mixture. At the end, *sodium azide* (Merck, CAS#26628-22-8) was added to the liquid and stirred with a glass rod as much as possible to obtain an almost uniform solution. Coconut oil was added to the solution to reach of a final volume of 10 mL. Eleven adult female mixed breed dogs (2-3 years old) were selected, weighing 18 ± 1.54 kg. Appropriate standard food and antiparasitic drugs (Caniverm, 0.7 mg tablet/10 kg/PO; Bioveta, Czeck Republic) were administered for at least 2 weeks before study. All dogs were in early anestrus based on previous history of their proestrus and diestrus was confirmed with vaginal cytology. Interestrus intervals of all dogs were about 7 months. Dogs were randomly assigned into control (cabergoline) and treatment (estradiol benzoate + cabergoline) groups. On day 0 of study, a single dose of 0.01 mg/kg estradiol benzoate was administered intramuscularly in 6 dogs (treatment group) and dogs in control group did not receive any hormones at day 0. One week later, 5 μg/kg/day of cabergoline was administered orally for all the dogs until beginning of proestrus. Vaginal cytology and blood samples were collected twice a week (Fig 1). The staging of estrus cycle was performed based on type and percentage of different vaginal epithelial cells.[23] Blood sample was taken from jugular vein and transferred to laboratory within 1-2 hours and centrifuged (10 min at 500*g*). Serum was stored at −20°C until evaluation of serum prolactin. Time to proestrus, proestrus, estrus length and serum prolactin concentration were compared between 2 groups by *t*-test and ANOVA with MedCalc version 12.1.4.0 software.

Results

The results of estrus cycle monitoring with vaginal cytology in the treatment and control groups is presented in Table 1. Dog number 11 from control group was removed from the study because of digestive disorders and diarrhea. We did not observe any side effects following estradiol benzoate and cabegoline administration. Two dogs (dog 4 from treatment group and dog 7 from control group) were in proestrus by day 7 and before administration of cabergoline and they were in prostrus by day 11. All of the dogs in treatment groups and dog 7 from control group were in proestrus by day 11. In day 14, all of the dogs were in estrus except dog 5 (treatment group) and dogs 8 and 9 (control group) which were in proestrus and dog 10 that was still in anestrus. By day 18, all of the dogs in treatment group were in estrus but, in control group dog 8 and 9 continued their proestrus and dog 10 remained in anestrus phase. Dogs 1, 2, 5, and 6 from treatment group and dog 7 from control group were in diestrus in day 21. Dogs 3 and 4 continued their estrus and dogs 8 and 9 were in estrus and dog 10 was in anestrus after 21 days cabergoline treatment. By day 25, all of the dogs were in diestrus except dogs 8 and 9 from control group. Finally, dog 10 from cabergoline treatment group was in estrus at day 25.

Average time to the onset of proestrus in treatment and control groups was 10.33 ± 4.2 and 15 ± 7.5 days, respectively (Table 2). Despite the apparent difference, it was not statistically significant

Table 1
Changes in Estrous Cycle Phases of Bitches in Control (Cabergoline) and Treatment (Estradiol Benzoate and Cabergoline) Groups

Group	Sampling Time (d)						
	0	7	11	14	18	21	25
Treatment (Dog 1)	A	A	PE	E	E	D	D
Treatment (Dog 2)	A	A	PE	E	E	D	D
Treatment (Dog 3)	A	A	PE	E	E	E	D
Treatment (Dog 4)	A	PE	PE	E	E	E	D
Treatment (Dog 5)	A	A	PE	PE	E	D	D
Treatment (Dog 6)	A	A	PE	E	E	D	D
Control (Dog 7)	A	PE	PE	E	E	D	D
Control (Dog 8)	A	A	A	PE	PE	E	E
Control (Dog 9)	A	A	A	PE	PE	E	E
Control (Dog 10)	A	A	A	A	A	A	PE
Control (Dog 11)	–	–	–	–	–	–	–

A, anestrus; D, diestrus; E, estrus; PE, proestrus.

($P = .08$). Average time to the onset of estrus in treatment group was 14.67 ± 5.9 days and it was 18.67 ± 10.8 days in control group. This difference was statistically significant ($P = .03$). The first observation of cytologic changes (related to each phase) was considered as the beginning of that stage. Average length of proestrus in treatment and control groups was 5.33 ± 2.2 and 8 ± 4.6 days, respectively. This difference was statistically significant ($P = .03$). The average length of estrus in dogs treated with estradiol benzoate was 8.57 ± 3.5 days and it was 8 ± 4.6 days in cabergoline treated dogs. It was not significant between groups ($P = .36$).

Changes in mean prolactin concentration, before and after cabergoline administration were plotted for each group in Fig 2. Changes of prolactin concentration (regardless of the groups) were significant over the time ($P < .05$). It means that cabergoline has decreased significantly at day 11 of the study or the fourth day after cabergoline administration ($P < .001$). However, difference of prolactin concentration was not statistically significant between control and treatment groups. The effect of cabergoline administration on prolactin concentration was not affected by estradiol benzoate in treatment group ($P = .4$).

Discussion

Estradiol benzoate injection before cabergoline treatment induced earlier and much more synchronized estrus than cabergoline in the bitches. Duration of proestrus and estrus phases was as good as normal cycles in both groups.

In this study, the proestrus started earlier (10.33 ± 4.2) in treatment than control group (15 ± 7.5 days). Phase of anestrus, hormones and breed affect time to onset of proestrus following induction of estrus protocols. Verstegen et al considered anestrus stage for induction of estrus with cabergoline in Beagle dogs. In almost all dogs proestrus occurred between days 4 and 30 of treatment. In the early and midanestrus groups, proestrus occurred earlier than late anestrus dogs.[13] Low dose of cabergoline (0.6 μg/kg/day) has been used for induction of estrus and ovulation in fertile dogs. Fertile estrus was induced during different stage of anestrus, economically. Estrus was induced 23.6 ± 14.33 (recommended dose) and 24.4 ± 14.31 days post onset of experiment (low dose).[11] So, dose of cabergoline did not affect induction of estrus response and time to onset of proestrus. In another experiment, the effect of testosterone therapy and dog breed was studied in induction of estrus with cabergoline. Dogs treated with testosterone showed proestrus symptoms (bleeding) for an average of 12.6 days (days 5-25) after cabergoline