

SHORTENING OF INTEROESTROUS INTERVAL IN DOGS USING CABERGOLINE AT MID ANOESTRUS

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Dopamine agonists were attempted for induction of oestrus in dogs with primary and secondary anoestrus. However, reports on the use of dopamine agonists in reducing the interoestrous interval in dogs are limited. The present study assessed the efficiency and safety of dopamine agonist, cabergoline in reducing the interoestrous interval in dogs by inducing oestrus at mid anoestrous stage. Twenty two healthy dogs that completed 120 ± 3 days following the previous proestrus were randomly allotted to treatment and control groups. Cabergoline @ $5\mu\text{g}/\text{kg}$ b.wt / day orally was given until onset of proestrus or until day 40, if no signs of proestrus were observed. All the treated dogs evinced proestrous bleeding within a mean period of 17.91 ± 4.21 days of start of treatment. A considerable shortening of IEI (82.82 ± 14.24 days) with an acceptable conception rate (72.72 %) was noticed in the treated dogs in contrast to an extension of the IEI (56.50 ± 9.18 days) with a conception rate of 66.67 per cent in control group

Keywords: Body Condition Score, Exfoliative vaginal cytology, Interoestrous interval.

Oestrous cycle in the dog is exceptional compared to other domestic animals in that an obligatory anoestrus follows proestrus, oestrus and dioestrus. However, a highly unpredictable period of obligatory anoestrus decreases the benefits of a commercial dog breeding programme. Interoestrous interval ranges normally from 5 to 11 months and averages 7 months in both fertile and non bred cycles in dogs (Verstegen *et al.*, 1999). Interoestrous interval as long as 11 months creates problems in devising an efficient breeding plan. Reliable methods of fertile oestrus induction have been difficult to work out in the dog, because the factors regulating termination of anoestrus and the onset of a new oestrous cycle are not completely understood. However, the termination of anoestrus happens with increased gonadotropin secretion and or increased sensitivity to GnRH (Verstegen *et al.*, 1999).

Feldman and Nelson (1996) reported that ninety days could be arbitrarily considered to be the duration of both follicular and luteal phases. Prolactin appears to play a role in the canine interoestrous interval, possibly by affecting gonadotropin secretion and/or ovarian responsiveness to

gonadotropins (Beijerink *et al.*, 2004).

Though administration of dopamine agonists like bromocryptine, metergoline and cabergoline were reported to terminate an abnormally prolonged anoestrus in normal ovarian cycles of dogs, its efficiency in terminating the obligatory anoestrus in normal ovarian cycles of dogs are scarce. The present study was hence undertaken to assess the efficiency and reliability of cabergoline in inducing fertile oestrus during the anoestrous stage of the normal ovarian cycle in dogs.

Materials and Methods

The study was conducted during a nine month period (August 2017 to May 2018) at University Veterinary Hospital, Kokkalai Thrissur, KVASU in clinically healthy, non-pregnant dogs of medium sized breeds that completed 120 ± 3 days following the preceding proestrus. Dogs aged 2 to 6 years with a Body Condition Score (BCS) of not less than four of a nine point scale were selected for the study. Anoestrous phase of the oestrous cycle was confirmed based on absence of intermediate and superficial cells in vaginal smears further confirmed by a serum progesterone level less than $1\text{ng}/\text{ml}$. Twenty two dogs that completed 120 ± 3 days of preceding proestrus and confirmed to be in anoestrous phase were randomly allotted to treatment (GI) and control groups (GII).

Part of M.V.Sc. Thesis

Serum progesterone (P4) and prolactin (PRL) were analysed before and after treatment where progesterone was analysed by ELISA (Enzyme Linked Immune Sorbent Assay) technique and prolactin was analysed by ELFA (Enzyme Linked Fluorescent Assay) technique. Breeding advice was provided as per exfoliative vaginal cytology (EVC), vaginoscopy and serum progesterone assay and breeding was done with healthy proven fertile male dogs. Treatment group was provided with cabergoline @ 5µg/kg bodyweight / day orally until onset of proestrus or until day 40 if no signs of proestrus were observed. Oestrous response rate, conception rate, gestation length, gestational accidents, and whelping rate were evaluated during the period of study. The data were statistically analysed by Independent t-test followed by Duncan multiple range test. Comparison of interoestrous interval before and after treatment was done by using paired t-test.

Oestrous response rate, conception rate and whelping rate were statistically analysed using chi square test for multiple proportion followed by Z-test for significance between two proportions. Statistical analysis was done using SPSS 24.0 Version.

Results and Discussion

The age of bitches in the study ranged between 2 to 6 years with GI and GII having a mean (\pm SE) of 3.68 ± 0.24 and 4.00 ± 0.23 years. The overall mean (\pm SE) interval calculated from last proestrual bleeding to treatment ranged between 117 to 123 days with a mean (\pm SE) of 120.18 ± 0.55 days. As per the haematological parameters and BCS, all selected animals were found to be healthy. The mean (\pm SE) progesterone (P4) level (ng/ml) in GI and GII was 0.42 ± 0.05 and 0.30 ± 0.07 , respectively and EVC revealed parabasal cells and small intermediates assuring that all animals were in anoestrous phase of the oestrous cycle. Similarly, Gobello *et al.* (2002) reported that female dogs with progesterone level below 1 ng/ml could be deemed to be in anoestrus. The mean serum prolactin level (ng/ml) in selected animals before treatment was <0.5 in

both GI and GII. This indicates the nonexistence of any hyperprolactinaemia in both treatment and control groups. Verstegen *et al.* (1999) also observed that serum prolactin concentrations in dogs at mid anoestrus was 1.6 ± 0.8 ng/ml and did not vary significantly in early, mid and late anoestrous. The difference in the levels obtained in these studies could be due to the variance in method of analysis.

The response to cabergoline administration in dogs of GI at 120.18 ± 0.55 days of preceding proestrus was cent percent as evinced by the display of proestrual bleeding by all the dogs. The mean duration from initiation of treatment to exhibition of proestrual bleeding was 17.91 ± 4.21 days. In the control group, only 6 animals (54.55%) exhibited oestrus throughout the nine month period of study. Mean (\pm SE) duration of proestrus was in GI was 10.36 ± 1.01 (range 6 to 17) and 10.45 ± 0.71 days (range 8 to 15) in GII (control). Mean (\pm SE) duration of oestrus in GI was 7.45 ± 0.39 (range 6 to 10) and 7.55 ± 0.41 days (range 5 to 9) in control group. Duration of proestrus and oestrus did not vary significantly ($p > 0.05$) between the groups. Average duration of proestrus and oestrus noticed correlates with the findings of earlier study by Gobello *et al.* (2004) who did not observe any difference in the duration of proestrus and oestrus in cabergoline induced and normally cycling dogs.

The behavioural characters such as intensity of proestrual bleeding, intensity of vulval oedema, interest towards male and tail deviation reflex were similar to spontaneous oestrous cycles. The mean (\pm SE) serum progesterone concentration (ng/ml) in GI and GII were 1.50 ± 0.06 and 1.87 ± 0.09 during proestrus, 5.06 ± 0.05 and 4.50 ± 0.38 during oestrus and increased to 16.08 ± 0.52 and 16.65 ± 0.55 during metoestrus. No significant difference was noticed between the groups in serum progesterone concentration during proestrus, oestrus, and metoestrus. Similar observations in serum progesterone concentration during proestrus, oestrus and metoestrus have also been reported by Sridevi and Veerapandian (2011). No detectable difference in the serum

prolactin level (<0.5 ng/ml) was observed before and after treatment in cabergoline treated dogs as well as in control animals. Similarly, no dissimilarity in the Anuclear Cell Index (ACI) was noticed between treated and control dogs both during proestrus and oestrus. The vaginoscopic appearance of vaginal mucosa during proestrus and oestrus also did not fluctuate between the groups. The hormonal, cytological and vaginal mucosal characteristics between the induced and spontaneous oestrus did not vary each other signifying that cabergoline could induce a normal oestrus and ovulation.

A decrease in interoestrous interval (82.82 ± 14.27 days) in cabergoline treated dogs (138.09 ± 3.14 days) in comparison to its preceding interoestrous interval (220.91 ± 3.58 days) was recorded. However, in control dogs, an elongation of interoestrous interval by 56.50 ± 9.18 days was noticed (228.00 ± 15.47 against 171.67 ± 13.90). The reduction in IEI in treatment dogs was significantly variable from the control dogs ($p < 0.05$). The termination of anoestrus with cabergoline in the present study was not related to the role of prolactin and progesterone as both the hormones were lower at the start of treatment. The response could be attributed to the observations by Beijerink *et al.* (2004) that dopamine agonists did not induce a follicular phase by prolactin suppression, but rather by other direct or indirect dopaminergic effects, like increased FSH secretion. Similarly Spattini *et al.* (2007) observed an increase in LH concentration without an effect on plasma prolactin level on administration of cabergoline in anoestrous bitches.

Conception rate among the animals that exhibited oestrus following treatment was 72.72 per cent (8/11). In the control group, the conception rate was 66.67 per cent (6/11) among the dogs that exhibited oestrus during the 9 month period of study. In GI, one abortion was recorded and in control group, no gestational accidents were evident. Hence, the whelping rate was 63.64 per cent (7/11) and 66.67 per cent (4/6) respectively in treated and control groups. Gestation length in treatment and control group was 60.57 ± 0.84 and 60.25 ± 0.90 days respectively which also shows no significant difference. Average litter size in treatment group was 3.86 ± 0.46 and in control group, it was 4.56 ± 0.42 . The neonatal survival rate in treatment and control group was 84.38 and 84.90 percent respectively. Side effects like vomiting, nausea and anorexia was noticed in 27 percent of treated animals even though, the drug was provided along with the food. However, with continuation of therapy, the side effects subsided on adaptation.

The induced oestrous cycle in cabergoline treated animals was normal, based on vaginal cytology and evidence of ovulation. Duration and physical characteristics were similar to natural oestrous cycle in control group. The present study confirmed the efficiency of $5 \mu\text{g}/\text{kg}$ b.wt cabergoline in shortening the interoestrous interval in dogs when administered during mid anoestrus stage. A normal oestrus and fertile ovulation was also assured as evident by normal duration of proestrus and oestrus and with a satisfactory conception rate following breeding in induced oestrus.

Table 1. Comparison of the efficacy of a treatment protocol with control group

Sl. No.	Parameters	Treatment Group (I)	Treatment Group (II)	P value
1.	Serum P4(ng/ml) (anoestrus)	0.42 ± 0.05	0.30 ± 0.07	0.648 ^{ns}
2.	Serum PRL(ng/ml) (anoestrus)	<0.5	<0.5	----
3.	Treatment duration (days)	17.91 ± 4.21	N.A.	----
4.	Oestrous response rate (%)	100 ^a	54.5 ^b	0.002*
5.	Duration of proestrus (days)	10.36 ± 1.01	10.45 ± 0.71	0.874 ^{ns}

6.	Duration of oestrus (days)	7.45 ±0.39	7.55 ±0.07	0.874 ^{ns}
7.	Serum PRL(ng/ml) (proestrus)	<0.5	<0.5	-----
8.	Serum P4(ng/ml) (proestrus)	1.50 ±0.06 ^b	1.87 ±0.09 ^a	0.002*
9.	Serum P4(ng/ml) (oestrus)	5.06 ±0.50	4.50 ±0.38	0.389 ^{ns}
10.	Anuclear cell index (oestrus)	52.45 ±2.94	49.27 ±2.40	0.412 ^{ns}
11.	Serum P4(ng/ml) (metoestrus)	16.08 ±0.52	16.65 ±0.55	0.459 ^{ns}
12.	Conception rate (%)	72.73(8/11)	66.67(4/6)	0.796 ^{ns}
13.	Whelping rate (%)	63.64 (7/11)	66.67(4/6)	0.900 ^{ns}
14.	Litter size	3.86 ± 0.46 ^b	4.56 ± 0.42 ^a	0.039*
15.	Gestation length	60.57± 0.84	60.25 ± 0.90	0.800
16.	Neonatal survival rate(%)	84.38	84.90	-----
17.				

Means with different superscripts differs significantly (p<0.05)

NA means not applicable

Table 2. Inter-oestrous interval (IEI)

Parameter	Treatment group (GI)	Control group (GII)	p value
IEI ₁	220.91±13.58 ^a	171.67 ± 13.9 ^b	0.032*
IEI ₂	138.09±3.14 ^b	228 ±15.47 ^a	<0.001**
Reduction of IEI(days)	82.82± 14.27 ^a	-56.50 ±9.18 ^b	<0.001**

Means with different superscripts differs significantly (p<0.05)

IEI₁= preceding IEI

IEI₂= Current IEI

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