



Shortening of dioestrus in female dogs using cloprostenol sodium at different stages of dioestrus[#]

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Abstract

A study was conducted in 18 healthy, fertile, non-pregnant, dioestrous female dogs to evaluate the efficacy of PGF_{2α} analogue (cloprostenol sodium) in shortening the dioestrus. Bitches that were diagnosed non-pregnant by trans-abdominal ultrasonography, on day 30 of breeding and their dioestrous stage confirmed by serum progesterone assay, were randomly allotted to three groups of six bitches each. Group I and II bitches were treated with cloprostenol sodium @ 2.5 µg/kg b. wt., subcutaneously, thrice at 48 h interval, beginning on day 30 and 45 of last breeding, respectively. Group III animals were assigned as control and were left untreated. Fifty days from first injection of cloprostenol, serum progesterone assay was carried out among the animals in Group I and II whereas the same was done among Group III bitches on day 90 after last breeding. Among Group I and II, mean serum progesterone concentrations were 0.93 ± 0.04 and 0.87 ± 0.01 ng/mL, respectively whereas the level was 1.72 ± 0.9 ng/mL among control group; highly significant difference ($p < 0.01$) existed between control and treatment groups. The present study indicated that administration of PGF_{2α} analogue during dioestrus enhances the pace of luteal regression among non-pregnant bitches.

Keywords: Dioestrus, PGF_{2α}, luteolysis, non-pregnant, bitches

Running title: Shortening of dioestrus in female dogs using cloprostenol sodium

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Canines are non-seasonal, monoestrous, polytocous and spontaneous ovulators (Concannon, 2011). Their prolonged dioestrus and obligatory anoestrus extend the inter-oestrous interval (IEI) up-to 5- 12 months, eventually lowering the profitability of canine breeding industry, due to lesser frequency of puppy harvest. Further, prolonged gestational phase predisposes the bitches to severe systemic diseases like cystic endometrial hyperplasia-pyometra complex (CEH-P) and other less severe conditions like pseudo-pregnancy (Johnston *et al.*, 2001). The present study focused on the efficiency of synthetic PGF_{2α} analogue, cloprostenol sodium, when administered at mid- dioestrus and 15 days later, to shorten the period of prolonged dioestrus in non-pregnant bitches, so as to reduce the IEI as well as risk of pathological conditions.

Materials and methods

Eighteen apparently healthy and fertile bitches, aged 2-8 years and diagnosed non pregnant by trans-abdominal ultrasonography on day 30 from last breeding, were selected for the study. The health status of all the bitches was validated by haematological analysis, peripheral blood smear and wet film examination as well as microscopical examination of faecal sample. Serum progesterone assay was done on the same day, to confirm dioestrus, by chemiluminiscense immune-assay (CLIA) as per manufactures instructions (Roche, cobas e 411 analyzer, USA) and expressed as ng/mL. The selected dogs were randomly allotted into three groups and treated as presented in Table 1.

Progesterone assay was repeated 50

days from first injection of cloprostenol among the animals in Group I and II whereas the same was done on day 90 after last breeding, among Group III bitches. The data obtained were tabulated and analysed statistically (Snedecor and Cochran, 1994) using one-way ANOVA and SPSS version 21.

Results and discussion

Serum progesterone assay during mid-dioestrus, on the day of selection

Mean serum progesterone concentration on day 30 from last breeding was 25.52 ± 2.14 , 23.64 ± 2.41 and 22.47 ± 2.46 ng/mL, respectively in group I, II and III. The values did not differ significantly ($p > 0.05$) between groups.

Similar values of dioestral serum progesterone concentration of 15-80 ng/mL were reported by Concannon (2011). Ucar *et al.* (2018) recorded serum progesterone concentration of 32.62 ± 2.42 ng/ml during mid dioestrus. Dioestrus has been referred to as the period of highly active *corpora lutea* (CL) which secretes large amounts of progesterone (Noakes *et al.*, 2019). Observations in the present study indicate the existence of functional CL in the animals under study.

Serum progesterone assay after cloprostenol administration

Mean serum progesterone concentration after 50 days of cloprostenol administration among the bitches of treatment groups and 90 days after last breeding (late dioestrus) in control animals are presented in Table 2.

Table 1. Treatment protocol using cloprostenol sodium in shortening of dioestrus in bitches

Group (n=6)	Day of treatment	Treatment protocol
I	30 th day since last breeding date (Day 0)	Cloprostenol sodium* @ 2.5µg/kg b.wt., s/c, three injections at 48 h interval
II	45 th day since last breeding date (Day 0)	cloprostenol sodium (Inj. Clostenol – 2mL) @ 2.5µg/kg b.wt., s/c, three injections at 48 h interval
III	Control	Animals were left untreated

*Inj. Clostenol – 2 mL (cloprostenol sodium 250µg/mL): Zydus Animal Health, Cadila Healthcare Limited, Ahmedabad

Table 2. Serum progesterone profile of the bitches under study after prostaglandin therapy /90th day of last breeding among control animals

Animal no.	Progesterone values (ng/mL)		
	Group I (day 80)	Group II (day 95)	Group III (day 90)
1	0.94	0.82	2.02
2	0.92	0.93	1.78
3	0.9	0.88	1.73
4	0.79	0.86	1.86
5	1.07	0.86	1.54
6	0.86	0.89	1.41
Mean ± SE	0.93 ± 0.04 ^b	0.87 ± 0.01 ^b	1.72 ± 0.9 ^a

Means with different superscripts differ significantly between columns

Mean serum progesterone concentrations of 0.93 ± 0.04 and 0.87 ± 0.01 ng/mL in treatment group I and II, respectively indicates that the bitches have entered in to anoestrus. Okkens and Kooistra (2006) and Kustritz (2012) reported that the bitches were considered to be in anoestrous phase when the serum progesterone concentration reached ≤ 1 ng/mL. Control animals maintained significantly higher serum progesterone (1.72 ± 0.9 ng/mL) than the treated animals, indicating that although serum progesterone concentration has drastically reduced from those recorded on day 30 of cycle (22.47 ± 2.46 ng/mL), they had not entered into anoestrus phase. The dioestrous phase in non-pregnant animals may be extended up-to 55 to 100 days and could be associated with varying degree of decline in plasma progesterone levels (Groppetti *et al.*, 2010).

Observation in the present study signifies the efficacy of prostaglandin in inducing luteolysis. Prostaglandins are proven to be more efficient during mid to later stages of dioestrus (Wichtel *et al.*, 1990). Kowalewski *et al.* (2009) reported that an increase in expression of PGF_{2 α} receptors during early to late dioestrus enables exogenous PGF_{2 α} to act on the CL for induction of luteolysis. Previous study (Ucar *et al.*, 2018) reported that multiple low doses of prostaglandins are more effective in inducing luteolysis in bitches. Drop in serum progesterone to basal levels among the treated bitches in the present study corroborates with the findings of Romagnoli (2017) who reported that administration of cloprostenol @ 2.5 μ g/

kg b wt. subcutaneously, three times at 48 h intervals was 100 per cent effective in inducing luteolysis when used after day 30 of dioestrus.

Slow dip in serum progesterone concentration noticed among untreated bitches in the present study is in accordance with Papa and Kowalewski (2020) who reported that gradual decline in serum progesterone levels occurs from day 35 post-ovulation onwards in non-pregnant animals.

Conclusion

The results of the present study indicate that administration of cloprostenol has shortened diestrus phase and animals have entered into anoestrus at a faster pace than the normal progression to anoestrus in untreated bitches.

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Conflict of interest

The authors declare that they have no conflict of interest.

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