



INDUCTION OF FERTILE OESTRUS IN BITCHES USING A SUSTAINED RELEASE PREPARATION OF A GnRH ANALOGUE – LEUPROLIDE ACETATE*

B. Bibin Becha¹ and K. N. Aravinda Ghosh²

Department of Animal Reproduction, Gynaecology & Obstetrics
College of Veterinary & Animal Sciences
Mannuthy - 680 651, Thrissur, Kerala

Abstract

Anoestrus bitches were treated with a sustained release preparation of leuprolide acetate @ 100 µg/kg body weight followed by gonadorelin @ 3 µg/kg body weight on the first day of induced oestrus. Bitches in natural proestrus were studied as controls. All the treated animals evinced proestrous bleeding by 4.67±0.21 days and oestrus by 12.67±0.49 days of treatment. The duration of proestrous bleeding was 6.67±0.56 days which was significantly shorter when compared to 8.67±0.42 days in controls. The intensity of proestrous bleeding and oestrus responses were pronounced in treated animals. There was no significant difference in the duration of oestrus, conception rate, gestation length and litter size between treated and control animals.

It is concluded that bitches in anoestrus could be induced to fertile oestrus reliably and rapidly using parenteral administration of a sustained release preparation of leuprolide acetate followed by gonadorelin, with a higher induction and conception rate with normal litter size.

Key words: Anoestrus, Induction of oestrus, Leuprolide acetate

Canines possess a unique reproductive biology. Sexual inactivity for several months after an oestrus is observed in non pregnant bitches due to prolonged dioestrus and anoestrus. The average inter-oestrus interval ranges from 5 to 12

months. Bitches with greater than average inter-oestrus interval and with delayed puberty create problems in formulating an efficient breeding programme.

Literature reveals the use of a variety of exogenous hormones and their combinations for induction of oestrus with varying results. Several regimes using gonadotropins (Wright, 1980; Renton *et al.*, 1981; Allen, 1982 and Simon, 1997) and their combinations with other hormones (Archbald *et al.*, 1980; Tsutsui *et al.*, 1982; Nakao *et al.*, 1985; Concannon, 1992 and Tsuda *et al.*, 1995) have been investigated. Most of the protocols gave poor results and suggested further improvement for clinical application.

Oestrus induction was obtained in bitches using pulsatile administration of GnRH using indwelling infusion catheters (Vanderlip *et al.*, 1987; Cain *et al.*, 1988 and Shille *et al.*, 1989). However the effort and expense in using battery operated portable pulsatile infusion pumps in bitches makes the method impractical in routine or clinical application.

The present study was undertaken to estimate the efficacy of a single parenteral administration of a sustained release preparation of a GnRH analogue – Leuprolide acetate in inducing oestrus with optimum fertility and fecundity in bitches.

Materials and Methods

Animals for the study consisted of six adult healthy anoestrus bitches and six healthy

* Part of M.V.Sc. thesis submitted by the first author to the Kerala Agricultural University, Thrissur

1. Assistant Professor

2. Professor & Head

bitches in natural oestrus. They belonged to the age group of 2 to 5 years with a history of at least one whelping. Anoestrus bitches were selected based on history and confirmed by vaginal cytology.

Anoestrus bitches were treated with a sustained release preparation of a GnRH analogue, Leuprolide acetate (Inj. Lupron depot*) at the rate of 100µg/ Kg. body weight intramuscularly and they were closely observed for the onset of vaginal bleeding. Proestrus and oestrus were confirmed by vaginal cytology. Proestrous bleeding and oestrus responses like intensity of vulval oedema, interest towards male and tail deviation reflex were graded according to their intensity. On the first day of behavioural and cytological oestrus, a potent GnRH agonist, gonadorelin (Inj. Fertagyl**) was administered at the rate of 3µg/ Kg. body weight intramuscularly.

All the bitches were allowed to mate twice with fertile males during oestrus based

on vaginal cytology. First mating was allowed when the vaginal cytological picture showed 80% superficial cells. Second mating was done when there was at least 10% increase in the number of superficial cells. Pregnancy diagnosis was carried out between 28 and 32 days after the first mating by transabdominal palpation. Gestation length was calculated as the duration between the day of first mating to the day of whelping. The litter size in both groups was also assessed.

Results and Discussion

All the treated bitches exhibited proestrous bleeding by 4.67 ± 0.21 days of injection of leuprolide acetate. Five out of six animals (83.3%) in treated group exhibited high intensity of proestrous bleeding (33.3% in control group) and high intensity of vulval oedema (50% in control group). One animal (16.7%) in treated group showed medium intensity of proestrous bleeding (66.7% in control group) and medium intensity of vulval oedema (33.3% in

Table 1. Proestrus and oestrus response in treatment and control group of animals

Group	No. of animals treated	Onset of proestrus after treatment (Days)	Duration of proestrus (Days)	Oestrus response (%)	Onset of oestrus after treatment (Days)	Duration of oestrus (Days)
Treatment	6	4.67 ± 0.21	6.67 ± 0.56^a	100 ^a	12.67 ± 0.49	8.00 ± 0.45^a
Control	6	NA	8.67 ± 0.42^b	100 ^a	NA	8.00 ± 0.45^a

Figures having different superscripts in a column differ significantly ($P < 0.05$)

control group). One animal (16.7%) in control group showed low intensity of vulval oedema.

Treated animals exhibited proestrous bleeding for a duration of 6.67 ± 0.56 days compared to 8.67 ± 0.42 days in control group. All animals exhibited oestrus according to behavioural and cytological findings. All the treated animals exhibited oestrus by 12.67 ± 0.49 days of treatment. The duration of oestrus in treated and control animals were 8.00 ± 0.45 days.

Oestrus response was assessed by intensity of vulval oedema, interest towards male and tail deviation reflex. High intensity of vulval oedema was shown by 83.3% animals compared to 50% animals in control group.

Five out of six (83.3%) animals in treated group showed high interest towards male compared to 66.7% animals in control group. One animal (16.7%) in treated group showed medium interest towards male compared to 33.3% animals in control group. All the treated animals exhibited tail deviation reflex compared to 83.3% animals in control group. One animal (16.7%) in control group didn't exhibit tail deviation reflex.

The proestrus and oestrus responses and their intensity in treated and control group of animals are presented in tables 1 and 2.

A conception rate of 83.3% was obtained in treated and control animals. Treated animals had a gestation length of

* Inj. Lupron depot (Takeda Chemical Industries Ltd., Osaka, Japan) – a sustained release preparation of leuprolide acetate, a synthetic nonapeptide analogue of naturally occurring GnRH: 3.75mg / vial

**Inj. Fertagyl (Intervet, Holland) – an aqueous solution of gonadorelin, a synthetic GnRH: 100 µg / ml

Table 2. Intensity of proestrus and oestrus in treatment and control groups

Parameter	Score	Treatment group (n=6)	Control group (n=6)
Intensity of proestrual bleeding	High	5(83.3%)	2(33.3%)
	Medium	1(16.7%)	4(66.7%)
	Low	—	—
Intensity of vulval oedema	High	5(83.3%)	3(50%)
	Medium	1(16.7%)	2(33.3%)
	Low	—	1(16.7%)
Interest towards male	High	5(83.3%)	4(66.7%)
	Medium	1(16.7%)	2(33.3%)
	Low	—	—
Tail deviation reflex	Present	6(100%)	5(83.3%)
	Absent	—	1(16.7%)

62.5±0.51 days compared to 62.0±0.71 days in control group. A litter size of 5.6±0.75 (4 to 8) was produced by treated animals compared to 5.6±1.17 (2 to 9) in control group. Toxic symptoms of hyperoestrogenism and foetal resorptions or abortions due to premature regression of the induced corpora lutea were not observed in this study. The findings are summarized in table 3.

In this study, fertile oestrus could be induced in 83.3% animals with a single intramuscular injection of a depot preparation of a GnRH analogue, Leuprolide acetate followed by a small dose of gonadorelin at the induced oestrus. Treated animals exhibited proestrual bleeding in 4.67±0.21 days and oestrus in 12.67±0.49 days of treatment. The consistent and rapid onset of proestrus in all animals suggests that there is increased follicular activity in the ovaries following treatment. In a similar study, a proestrus response of 100% in GnRH treated animals was also reported by Inaba *et al.* (1998), but only lowered results were obtained by Vanderlip *et al.* (1987), Shille *et al.* (1989) and Concannon (1989) in their oestrus induction trials using GnRH. The duration of proestrus

was much shorter in GnRH treated animals when compared to control animals which were in agreement with the earlier works of Inaba *et al.* (1998). This might be due to increased stimulation of ovaries leading to high production of oestrogens resulting in feedback inhibition of pituitary hormones.

Intensity of proestrual bleeding, vulval oedema, interest towards male and tail deviation reflex were high in treated animals. There was significant reduction in the duration of proestrus in treated animals, but there was no significant changes in the duration of oestrus between treated and control animals indicating a normal hormonal pattern in induced and natural oestrus.

The pregnancy rate of 83.3% in oestrus induced animals was similar to the observations of Inaba *et al.* (1998) using GnRH for oestrus induction. But the results were higher compared to the results of Vanderlip *et al.*, 1987, Cain *et al.*, 1989 and Concannon, 1989 using GnRH for oestrus induction.

It was concluded that oestrus induction in bitches using a sustained release preparation of leuprolide acetate (GnRH

Table 3. Conception rate, gestation length and litter size in treatment and control animals

Group	Number of animals treated	Number of animals conceived	Conception rate (%)	Gestation length (Days)	Number of animals whelped	Litter size
Treatment	6	5	83.3	62.5±0.51	5	5.6±0.75
Control	6	5	83.3	62.0±0.71	5	5.6±1.17

analogue) was reliable and rapid with higher conception rate and litter size similar to natural oestrous cycles. This single dose regimen was more effective and convenient when compared to other oestrus induction regimens using GnRH where use of continuous infusion or pulsatile infusion pumps was necessary. No adverse effects or toxic symptoms were evinced by the treatment regimen adopted in this study.

Acknowledgement

The authors thank the Dean, College of Veterinary & Animal Sciences, Mannuthy for providing facilities for the research work. The authors are highly thankful to M/s. Takeda Chemical Industries Ltd., Osaka, Japan for providing the drug samples for the study.

References

- Allen, W.E. 1982. Attempted oestrus induction in four bitches using pregnant mare serum gonadotrophin. *J. small Anim. Pract.*, **23**: 223-231.
- Archbald, L.F., Baker, B.A., Clooney, L.L. and Godke, R.A. 1980. Surgical method for collecting canine embryos after induction of estrus and ovulation with exogenous gonadotrophins. *Vet. Med.Small Anim. Clin.*, **75**: 228-233.
- Cain, J.L., Cain, G.R., Feldman, E.C., Lasley, B.L. and Stabenfeldt, G. 1988. Use of pulsatile intravenous administration of gonadotropin-releasing hormone to induce fertile estrus in bitches. *Am. J. Vet. Res.*, **49**: 1993-1996.
- Cain, J.L., Lasley, B.L., Cain, G.R., Feldman, E.C. and Stabenfeldt, G.H. 1989. Induction of ovulation in bitches with pulsatile or continuous infusion of GnRH. *J. Reprod. Fert. Suppl.*, **39**: 143-147.
- Concannon, P.W. 1989. Induction of fertile oestrus in anoestrous dogs by constant infusion of GnRH agonist. *J. Reprod. Fert., Suppl.*, **39**: 149-160.
- Concannon, P.W. 1992. Methods for rapid induction of fertile oestrus in dogs. In: Kirk's Current Veterinary Therapy: Small Animal Practice; eds. Kirk, R.W. and Bonagura, J.D., W.B. Saunders Company, Philadelphia. pp: 960-963.
- Inaba, T., Tani, H., Gonda, M., Nakagawa, A., Ohmura, M., Mori, J., Torii, R., Tamada, H. and Sawada, T. 1998. Induction of fertile estrus in bitches using a sustained release formulation of a GnRH agonist (Leuprolide acetate). *Theriogenology* **49**: 975-982.
- Nakao, T., Aoto, Y., Fukushima, S., Moriyoshi, M. and Kawata, K. 1985. Induction of estrus in bitches with exogenous gonadotrophins and pregnancy rate and blood progesterone profiles. *Jap. J. Vet. Sci.*, **47**: 17-24.
- Renton, J.P., Munro, C.D., Heathcote, R.H. and Carmichael, S. 1981. Some aspects of the aetiology, diagnosis and treatment of infertility in the bitch. *J. Reprod. Fert.*, **61**: 289-294.
- Shille, V.M., Thatcher, M.J., Lloyd, M.L., Miller, D.D., Seyfert, D.F. and Sherrod, J.D. 1989. Gonadotrophic control of follicular development and the use of exogenous gonadotrophins for induction of oestrus and ovulation in the bitch. *J. Reprod. Fert. Suppl.*, **39**: 103-113.
- Simon, S. 1997. Hormonal induction of oestrus in bitches. *M.V.Sc. Thesis*. Kerala Agricultural University, Thrissur.
- Tsutsui, T., Shimizu, T., Shimizu, K. and Matsuzaki, M. 1982. Induction of oestrus and ovulation in the bitch - Application of PMSG and hCG. *Bulletin of the Nippon Veterinary and Zootechnical College*, **31**: 143-147.
- Tsuda, Y., Nakao, T., Moriyoshi, M. and Kawata, K. 1995. The induction of superovulation in the bitch with pregnant mare serum gonadotrophin and human chorionic gonadotrophin. *J. Reprod. Dev.*, **41**: 89-95.
- Vanderlip, S.L., Wing, A.E., Felt, P., Linkie, D., Rivier, J., Concannon, P.W. and Lasley, B.L. 1987. Ovulation induction in anoestrous bitches by pulsatile administration of gonadotrophin releasing hormone. *Lab. Anim. Sci.*, **37**: 459-464.
- Wright, P.J. 1980. The induction of oestrus and ovulation in the bitch using pregnant mare serum gonadotrophin and human chorionic gonadotrophin. *Aust. Vet. J.*, **56**: 137-140.

