SERUM PROGESTERONE PROFILE DURING DIFFERENT STAGES OF INDUCED AND NATURAL ESTROUS CYCLES IN BITCHES*

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ABSTRACT

Estrus was induced in twelve anestrus bitches using a sustained release preparation of leuprolide acetate and diethylstilbestrol. Bitches with GnRH regimen of estrus induction (n=6) showed a mean serum progesterone level of 0.72 ± 0.33 ng/ml on the first day of treatment, 0.28 ± 0.08 ng/ml on the second day of proestrus, 3.80 ± 1.07 ng/ml on the first day of estrus, 15.12 ± 3.73 ng/ml on the day of second mating and 17.92 ± 1.15 ng/ml on the tenth day of second mating. Bitches treated with diethylstilbestrol (n=6) showed a mean serum progesterone profile of 0.47 ± 0.27 ng/ml on the first day of treatment, 0.2 ng/ml on the second day of proestrus, 5.53 ± 0.66 ng/ml on the first day of estrus, 13.67 ± 2.84 ng/ml on the day of second mating and 15.87 ± 0.27 ng/ml on the tenth day of second mating. Animals in natural estrum (n=6) showed a mean serum progesterone level of 0.36 ± 0.12 ng/ml on the second day of proestrus, 3.56 ± 0.50 ng/ml on the first day of estrus, 8.12 ± 0.36 ng/ml on the day of second mating and 17.80 ± 0.58 ng/ml on the tenth day of second mating. In treatment groups, the serum progesterone levels raised quickly to higher levels during estrum when compared to control animals, but in all animals it reached to similar levels by metestrum. Retrospective studies revealed that ovulation occurred in GnRH treated and diethylstilbestrol treated animals by 8th to 11th day and 6th to 11th day from first day of proestrus respectively. Ovulation occurred in estrus induced animals earlier than that in control animals which is due to the shortened and pronounced proestral changes in treated animals. But in animals where estrus was induced using diethylstilbestrol, the ovulatory period was inconsistent which necessitates more frequent assessment of progesterone profile for timing of ovulation. GnRH treated animals, diethylstilbestrol treated animals and control animals had a conception rate of 83.3, 50 and 83.3% respectively with similar gestation length and litter size in all groups. It is concluded that serum progesterone profile studies can be employed for selecting good subjects for estrus induction treatment, timing of ovulation and to assess the normal progression of the estrous cycle in natural and induced estrous cycles.

Key words: Induction of estrus, Progesterone profile, Bitches.

INTRODUCTION

Dog breeding has emerged as a lucrative venture in animal husbandry. This has resulted in awareness for the scientific breeding according to the demands from breeders. Anestrus in bitches is a physiological phenomenon and maintaining anestrus bitches for prolonged periods makes the commercial dog breeding more expensive. Fecundity and prolificacy can be increased by shortening the naturally occurring or unusually prolonged anestrus in bitches by using a reliable estrus induction regimen. A variety of exogenous hormones and their combinations has been reported for induction of estrus in bitches with varying results (Renton *et al.* 1981; Allen, 1982; Nakao *et al.*, 1985; Vanderlip *et al.*, 1987; Concannon, 1992 and Tsuda *et al.*, 1995). Most of the regimens produced an alteration in the normal progression of the induced estrous cycle due to altered hormonal status in the animal which makes the timing of mating difficult in practical situations. These variations depends mainly on the hormonal status of the animal at the time of treatment, the type, dose, route, form, frequency and the efficacy of exogenous hormone used.

Unlike most of other reproductive hormones, serum progesterone can be monitored consistently during different stages of the estrous cycle which is useful in selecting good subjects for estrus induction, timing of ovulation and evaluating the progression of the estrus cycle after treatment for induction of estrum.

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In this study, the serum progesterone levels at various stages of the estrous cycle were analysed retrospectively in animals where estrus was induced using two estrus induction regimens and also in animals with naturally occurring estrous cycles.

MATERIALS AND METHODS

Twelve healthy anestrus bitches of 2 to 5 years of age with a history of at least one whelping and another six bitches in natural estrus were selected for this study. Estrus was induced in six anestrus bitches (Group A) with a single parenteral administration of a sustained release preparation of leuprolide acetate @ 100µg/Kg body weight followed by gonadorelin @ 3µg/Kg body weight on the first day of induced estrus. Six anestrus bitches (Group B) were treated with diethylstilbestrol @ 0.2mg/Kg body weight orally for nine days. Six bitches in natural proestrus (Group C) formed the control. All the bitches were allowed to mate with fertile males twice during estrus based on vaginal cytology. First mating was recommended when more than 60% of the exfoliated cells became superficial cells and the second mating was recommended when there was a 10 to 20% increase in the number of superficial cells in the vaginal cytology smear.

Blood samples were collected on the first day of treatment, second day of proestral bleeding, on the first day of induced estrus, on the day of second mating and tenth day of second mating from anestrus bitches (Group A & B). Blood samples were collected on the second day of proestral bleeding, on the first day of induced estrus, on the day of second mating and tenth day of second mating from Group C animals. Serum was separated and kept at –20°C till assaying. Serum progesterone levels were assayed quantitatively by competitive ELISA with streptavidin technology using Enzymun - Test progesterone kit (Boehringer Mannheim). The measuring range for the test was 0 to 30 ng/ml. A graph was prepared by plotting serum progesterone levels on respective days of sampling in all animals and the expected days of ovulation was predicted retrospectively by reading off the days against serum progesterone levels of 4 to 8ng/ml.

Pregnancy diagnosis was carried out between 28 and 32 days after first mating by trans-abdominal palpation. Gestation length was calculated as the duration between the day of first mating and the day of whelping. Conception rate and litter size were also assessed in all the three groups.

RESULTS AND DISCUSSION

Animals in Group A showed a mean serum progesterone level of 0.63 ± 0.28 ng/ml on the first day of treatment, 0.50 ± 0.23 ng/ml on the second day of proestrus, 3.37 ± 0.98 ng/ml on the first day of estrus, 12.63 ± 3.94 ng/ml on the day of second mating and 15.30 ± 2.78 ng/ml on the tenth day of second mating. Animals in Group B showed a mean serum progesterone level of 0.50 ± 0.19 ng/ml on the first day of treatment, 0.2 ng/ml on the second day of proestrus, 5.95 ± 0.62 ng/ml on the first day of estrus, 12.25 ± 2.46 ng/ml on the day of second mating and 13.05 ± 2.82 ng/ml on the tenth day of second mating. Animals in Group C showed a mean serum progesterone level of 0.37 ± 0.10 ng/ml on the second day of proestrus, 3.10 ± 0.61 ng/ml on the first day of estrus, 6.87 ± 1.29 ng/ml on the day of second mating and 15.10 ± 2.74 ng/ml on the tenth day of second mating.

There was no significant difference in progesterone levels on the first day of treatment between treatment groups, but there was significant difference in progesterone levels on the second day of proestrus and on the first day of estrus between treatment groups and between Group B and Group C. Significant difference ($P<0.05$) in progesterone levels were observed on the day of second mating between Group A and Group C and between Group B and Group C. There was no significant difference in progesterone levels on the tenth day of second mating between different groups. Similar findings of lower levels of serum progesterone were observed in anestrus bitches by Concannon, (1986) and Arnold *et al.* (1989). Very low level of progesterone during early proestrus was also observed by Concannon, (1986) and Tomaskovic *et al.* (1997). In treatment groups, the serum progesterone levels raised quickly to higher levels during estrum when compared to control animals, but in all groups it reached to similar levels by metestrum as observed by Verstegen *et al.* (1999).

Pregnant animals in Group A (n=5) showed a mean serum progesterone level of 0.72 ± 0.33 ng/ml on the first day of treatment, 0.28 ± 0.08 ng/ml on the second day of proestrus, 3.80 ± 1.07 ng/ml on the first day of estrus, 15.12 ± 3.73 ng/ml on the day of second mating and 17.92 ± 1.15 ng/ml on the tenth day of second mating. Pregnant animals in Group B (n=3) showed a mean serum progesterone profile of 0.47 ± 0.27 ng/ml on the first day of treatment, 0.2 ng/ml on the second day of proestrus, 5.53 ± 0.66 ng/ml on the first day of

estrus, 13.67 ± 2.84 ng/ml on the day of second mating and 15.87 ± 0.27 ng/ml on the tenth day of second mating. Pregnant animals in control group (Group C, n=5) showed a mean serum progesterone level of 0.36 ± 0.12 ng/ml on the second day of proestrus, 3.56 ± 0.50 ng/ml on the first day of estrus, 8.12 ± 0.36 ng/ml on the day of second mating and 17.80 ± 0.58 ng/ml on the tenth day of second mating.

Statistical analysis showed significant difference in progesterone levels during second day of proestrus between pregnant animals of Group B and Group C. There was significant difference in progesterone levels during first day of estrus between pregnant animals of Group A and Group B and between Group B and Group C. Significant difference in progesterone levels was observed during day of second mating between pregnant animals of Group B and Group C and between Group A and Group C. There was significant difference ($P<0.05$) in progesterone levels on tenth day after second mating between treatment groups and between Group B and Group C. Similar observations were recorded by Cain *et al.* (1989) and Verstegen *et al.* (1999).

At the onset of estrus, follicular cells luteinize and secrete significant amount of progesterone. At the time of ovulation, the serum progesterone concentrations were typically in the range of 4 to 10 ng/ml. Serum progesterone concentrations continued to increase drastically throughout estrus after ovulation and several weeks into diestrum due to the development of functional corpora lutea (Feldman and Nelson, 1996). Their findings are similar to the results of the present study.

In treatment groups, the serum progesterone levels raised quickly to higher levels during estrum when compared to control animals, which might be due to the continued action of exogenous hormones that supported the development of the corpora lutea. In treated and control animals, serum progesterone levels reached to similar levels by metestrum (Inaba *et al.* 1998).

Two animals in Group B did not respond to the estrus induction treatment. One treatment responded animal in each group did not conceive after matings with fertile males.

Estrus induced, non-pregnant animal in Group A showed a serum progesterone level of less than 0.2 ng/ml on the first day of treatment, 1.6 ng/ml on the second day of proestrus, 1.2 ng/ml on the first day of estrus, 0.2 ng/ml on the day of second mating and 2.2 ng/ml on the tenth day of second mating. Estrus induced, non pregnant animal in Group B showed serum progesterone levels of less than 0.2 ng/ml on first day of treatment and second day of proestrus, 7.2 ng/ml on the first day of estrus, 8.0 ng/ml on the day of second mating and 4.6 ng/ml on the tenth day of second mating. Non-pregnant animal in Group C showed serum progesterone levels of 0.4 ng/ml, 0.8 ng/ml, 0.6 ng/ml and 1.6 ng/ml on the second day of proestrus, on the first day of estrus, on the day of second mating and on the tenth day of second mating.

The inconsistent increase of serum progesterone in these animals indicated an abnormal progression of the estrous cycle. In all these animals, there was slight increase in serum progesterone levels which may be produced by luteinisation of follicular cells. The continued increase throughout estrus was not noticed which might be due to the failure of ovulation and subsequent formation of functional corpora lutea.

Concannon (1986) concluded that ovulation occurs in bitches when the serum progesterone level reaches 4 to 8 ng/ml. Based on this finding, the expected days of ovulation derived from the graph prepared by plotting progesterone levels on respective days of sampling in treated and control animals are presented in Table.

In GnRH analogue treated animals (Group A) ovulation occurred with a mean interval of 13th to 16th day from first day of treatment or 8th to 11th day from first day of proestrus or first to 4th day from onset of estrus. In diethylstilbestrol treated animals (Group B), ovulation was found to occur with a mean interval of 13th to 19th day from first day of treatment or 6th to 11th day from first day of proestrus or three days before to three days after the onset of estrus. Ovulation occurred in control animals (Group C) in a mean interval of 10th to 14th day from the first day of proestrus or from onset of estrus to four days later.

Ovulation was found to occur in estrus induced animals earlier than that in control animals which was probably due to shortened proestrus in treated animals. But in animals where estrus was induced using diethylstilbestrol, the ovulatory period was inconsistent (over a period of 6 days) which necessitates more frequent assessment of progesterone profile for timing of ovulation. But in all animals, the frequency of blood sampling for serum progesterone estimation has to be

increased (2 to 3 days interval) for more precise timing of ovulation (Concannon, 1986).

Five animals each (83.3%) in Group A and control group and three animals (50%) in Group B were found pregnant by 28 to 32 days of first mating by trans-abdominal palpation. Treated animals had a gestation length of 62.5±0.51 days (Group A) and 62.0±1.15 days (Group B) compared to 62.0±0.71 days in control group. A litter size of 5.6±0.75 (4 to 8) was produced in GnRH treated animals and 6.0±0.58 (5 to 7) was produced in diethylstilbestrol treated animals compared to 5.6±1.17 (2 to 9) in control group.

There was no significant difference in gestation length and litter size between treatment and control groups of animals. The pregnancy rate of 83.3% in Group A animals was similar to the observations of Inaba *et al.* (1998) using GnRH for estrus 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 estrus induction. Pregnancy rate of 50% in diethylstilbestrol treated animals (Group B) were much lower when compared to100% results obtained by Bouchard (1992) using the same treatment regimen.

Table : Predicted mean days of ovulation using serum progesterone profile

Animal	Expected days of ovulation		
	From first day of treatment	From first day of proestrus	From first day of estrus
Group A	13-16	8-11	1-4
Group B	13-19	6-11	-3 to 3
Group C	---	10-14	0-4

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