犬の開花期黄体に及ぼすPGF2α-analogue(Etiproston tromethamine)投与の影響

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FULL PAPER *Theriogenology*

Influence of a $PGF_{2\alpha}$-Analogue, Etiproston Tromethamine, on the Functional Corpus Luteum of Dogs

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ABSTRACT. To induce luteal regression-related abortion/delivery and treat pyometra in dogs, various $PGF_{2\alpha}$-analogues (PGAs) are administered, but a PGA most appropriate for clinical application in dogs, with a low incidence of side effects, is being investigated. In this study, we compared the effects of etiproston tromethamine (PGA-E), which has not been investigated in dogs, with those of cloprostenol (PGA-C), which is routinely used in dogs. A single dose of PGA-E at 100, 200, 400 or 800 μg or PGA-C at 12.5, 25, 50 or 100 μg was administered to beagles (n=5 per group) 25 days after ovulation, when the corpus luteum was in the functional phase. We compared the state of luteal regression by measuring plasma progesterone levels. As side effects, the incidences of salivation, vomiting, tachypnea, diarrhea and the drop in body temperature were investigated. In the 400-μg and 800-μg groups treated with PGA-E, the mean intervals from administration until luteal regression were 18.6 days and 31.2 days, respectively. In the dogs treated with 50 μg or more of PGA-C, luteal regression was noted 2 days after administration. The above side effects were observed for 3 hr after administration of PGA-E/PGA-C. In the dogs treated with 800 μg of PGA-E, the mean body temperature was 36.7°C 4 hr after administration; hypothermia persisted. PGA-E may be less useful than PGA-C for promoting luteal regression in dogs in clinical application.

KEY WORDS: canine, cloprostenol, corpus luteum, etiproston tromethamine, progesterone.

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To induce luteal regression-related abortion [2, 4, 5, 8, 18, 21, 22, 27] and delivery [9, 12, 14] and to treat pyometra [13, 16] in dogs, natural $PGF_{2\alpha}$ (PG) or its analogue (PGA) is used. As PG, dinoprost has been investigated [2, 4, 13, 16, 27]. As PGAs, several studies have investigated cloprostenol (PGA-C) [5, 10, 12], alphaprostol [21], $PGF_{2\alpha}$-1052 (16–3-chlorophenoxy-ω-tetranor-trans-Δ^2-$PGF_{2\alpha}$ methyl ester) [25, 26], fenprostalene [7–9, 14], and fluprostenol [10], but the types of PGA vary; it is being investigated which is most appropriate for promoting luteal regression in dogs. In clinical application, administration of PG or PGAs caused side effects such as salivation, vomiting, tachypnea, diarrhea, and drop in body temperature [2, 5, 7, 9, 25].

In this study, to determine a PGA most appropriate for promoting luteal regression in dogs, we investigated the effects of etiproston tromethamine, which is clinically applied in cattle [unpublished data Virbac, France, 1994] and pigs [24], but not in dogs. In addition, we compared changes in the blood progesterone (P_4) level after administration of those after administration of PGA-C [5, 10, 12], which is routinely used in dogs. The mechanism by which the corpus luteum in dogs is maintained remains to be clarified. In this study, we measured the plasma P_4 level after administration of PGA-E or PGA-C, and investigated the changes in LH and PRL in plasma to examine the relationship between luteal function and LH/PRL. We also investigated various side effects related to PGA administration. In addition, as administration of PG/PGAs advances subsequent estrus [18, 25], we investigated whether the interval between administration and subsequent estrus was shortened.

MATERIALS AND METHODS

Animal: A total of 47 female beagles ranging from 2.0 to 8.0 years of age and from 9 to 10 kg of body weight were used in this experiment. A commercially available dog food (Hill's Canine Maintenance, U.S.A.) was given once a day, and water was given 3 times a day (morning, noon and evening). This study was conducted in conformity with the animal study guidelines of Nippon Veterinary and Animal Science University.

Estimation of ovulation day: Estrus in the dogs was diagnosed by observing the vulva bleeding and the swelling of the vulva. From dogs showing signs of estrus, peripheral blood was collected once daily from the 6th day after the onset of vulval bleeding, as we previously reported [6]. The first day that a plasma P_4 level of 2 *ng*/m*l* or higher was recorded was regarded as the day of ovulation.

Administration of PGAs: A single dose of PGAs or physiological saline was subcutaneously administered to both experiment dogs and control dogs 25 days after ovulation. The doses of PGA-E (PROSTAVET®, containing 850 μg/m*l* of PGA-E, Sankyo Co., Ltd., Tokyo) were 100, 200, 400 and 800 μg (n=5 per group). The doses of PGA-C (RESIPRON-C®, containing 250 μg/m*l* of PGA-C, Teikoku Hormone Mfg. Co., Ltd., Tokyo) were 12.5, 25, 50 and 100 μg (n=5, 5, 6 and 5 per group, respectively). To the 6 control dogs, 1 m*l* of physiological saline was subcutaneously administered.

Measurement of plasma hormone levels: The influence of PGAs on luteal function was evaluated from changes in the plasma P_4 level. The influence of luteal regression on the pituitary hormones was evaluated from changes in LH and

PRL levels. To measure hormone levels, 3 m*l* of blood was collected 15, 20 and 25 (date of administration) days after ovulation, 4 hr after administration of PGAs, every day between 26 days and 28 days after ovulation, and at 3-day intervals until the plasma P_4 level decreased to the baseline (1 *ng*/m*l* or less), 29 days or more after ovulation. Immediately after collection, blood samples were centrifuged with a low- temperature centrifuge to isolate plasma, and stored at –40°C until hormone levels were measured.

Plasma P_4 was measured by an enzyme immunoassay method developed by Munro and Stabenfeldt [15]. The intra-assay coefficient of variation for samples was 8.8%, and the inter-assay coefficient of variation for the same pools was 13.9%. The sensitivity of this immunoassay method was 0.25 *pg*/well.

Plasma LH was measured by means of a double-antibody radioimmunoassay (RIA) method in accordance with the procedure described by Nett *et al.* [17] except that radiolabelled porcine LH (LER-778) and anti-porcine LH serum were used, as reported by the authors [11]. Purified canine LH (LER-1685) was used as the standard. The assay standard curve was done in duplicate, with 10 standard concentrations ranging from 0.098 to 50 *ng*/m*l*. Samples were assayed in duplicate in 100-μl aliquots. The intra-assay and inter-assay coefficients of variation were 9.3% and 7.0%, respectively. The minimum detectable concentration was 0.20 *ng*/m*l*.

Plasma PRL levels were determined by the homologous RIA method [1]. RIA determinations were made by a double antibody method. Highly purified cPRL (AFP-2451B: a generous gift from Dr. A.F.Parlow, Pituitary Center, UCLA, Los Angeles, CA) was used for iodination and for the standard curve. The assay standard curve was done in duplicate, with 10 standard concentrations ranging from 0.098 to 50 *ng*/m*l*. Samples were assayed in duplicate in 100-μl aliquots. The intra-assay and inter-assay coefficients of variation for the samples were 12.1 and 9.9%, respectively. The minimum detectable concentration of canine PRL was 0.20 *ng*/m*l*.

Side effects: As side effects, salivation, vomiting, diarrhea, and tachypnea were investigated 30 min and 1 hr after administration, and subsequently at 1-hr intervals until the disappearance of these side effects. Rectal temperature was measured 30 min and 1, 2, 4, and 16 hr after administration.

Recurrent estrus after administration of PGAs: The interval from administration of PGAs until recurrent estrus was compared among the PGA-E group, the PGA-C group, and the control group.

Statistical analysis: Data obtained in this study were analyzed by Student's *t*-test, and a significance level lower than 5% was defined as significant.

RESULTS

Changes in plasma hormones after administration of PGAs: The changes in plasma P_4 and PRL levels in the 4 dose groups treated with PGA-E or PGA-C and the control group are shown in Figs. 1 and 2 (mean ± SE).

Changes in the plasma P_4 level after administration of PGAs: The plasma P_4 levels in the 100-μg and 200-μg groups treated with PGA-E decreased 4 hr and 1 day after administration, respectively, but slightly increased 2 days after administration, and then gradually decreased. The plasma P_4 levels decreased to the baseline 31.2 ± 3.7 days (21–38 days) and 18.6 ± 4.4 days (6–27 days) after administration of PGA-E, respectively. In the high-dose groups (400- and 800-μg), the plasma P_4 levels decreased to the baseline 13.8 ± 4.6 days (3–27 days) and 8.6 ± 4.3 days (2–18 days) after administration of PGA-E, respectively.

The plasma P_4 levels in the 12.5-μg and 25-μg groups treated with PGA-C decreased to the baseline 9.6 ± 2.0 days (6–15 days) and 3.3 ± 0.6 days (2–6 days) after administration, respectively. In the 50-μg and 100-μg groups treated with PGA-C, the plasma P_4 levels decreased to the baseline 2 days after administration.

Changes in the plasma LH level after administration of PGAs: There were no marked PGA-related changes in the plasma LH level at any dose in the PGA-E or PGA-C groups.

Changes in the plasma PRL level after administration of PGAs: The plasma PRL levels in the 4 dose groups treated with PGA-E or PGA-C reached a peak 4 hr or 1 day after administration of PGAs, respectively. The mean peak values of PRL were 2.69, 3.18, 2.74 and 3.54 *ng*/m*l* in the 100-μg, 200-μg, 400-μg, and 800-μg groups treated with PGA-E, respectively, which gradually decreased. The mean intervals required until these values returned to the pretreatment values were 3, 9, 15 and 18 days, respectively, with a dose dependency.

In the control group, there were no marked changes in the plasma PRL level after administration of physiological saline.

The mean peak values of PRL were 3.38, 5.31, 5.15 and 5.67 *ng*/m*l* in the 12.5-μg, 25-μg, 50-μg and 100-μg groups treated with PGA-C, respectively. In each group, the value returned to the pretreatment value 12 days after the peak was obtained. Subsequently, the levels of PRL were similar to the pretreatment values until 40 to 55 days after ovulation.

Side effects related to administration of PGAs: Side effects, such as salivation, diarrhea, vomiting and tachypnea, were observed in the 10 dogs treated with 400 or 800 μg of PGA-E, and these symptoms were most marked 1 hr and 30 min after administration, but disappeared 3 hr after administration. Among the dogs treated with 100 or 200 μg of PGA-E, no animal in the 100-μg group showed salivation, but the other side effects were observed in 3 of the 5 dogs in each group. These symptoms disappeared 2 hr after administration.

In the 50-μg and 100-μg groups, salivation did not occur in 1 of the 5 dogs, but diarrhea, vomiting and tachpnea were observed in all dogs. The side effects were most marked 1 hr and 30 min after administration, but disappeared 3 hr after administration. In the 12.5- μg and 25-μg groups, no side effect occurred in 2 and 3 of the 5 dogs, respectively,

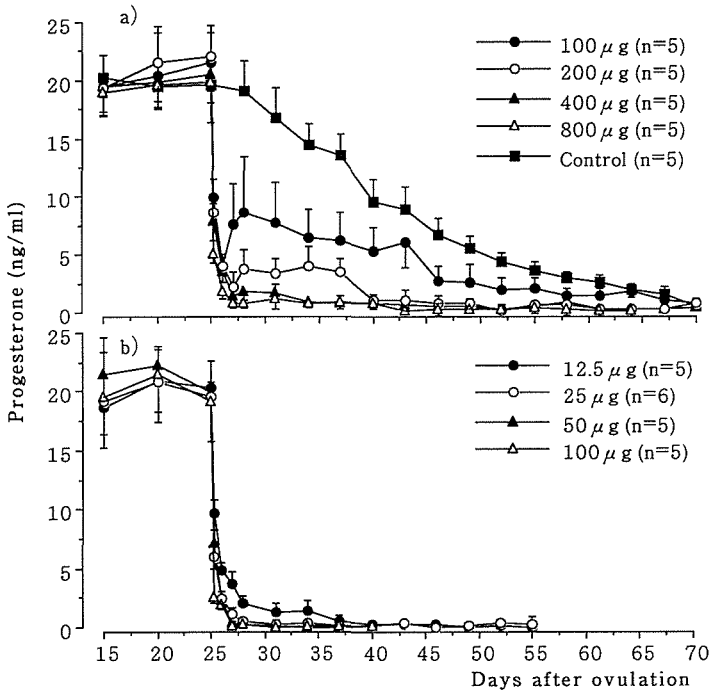

Fig. 1. Changes in the plasma P_4 level (means ± SE) after PGA-E (a) and PGA-C (b) administration at 25 days after ovulation.

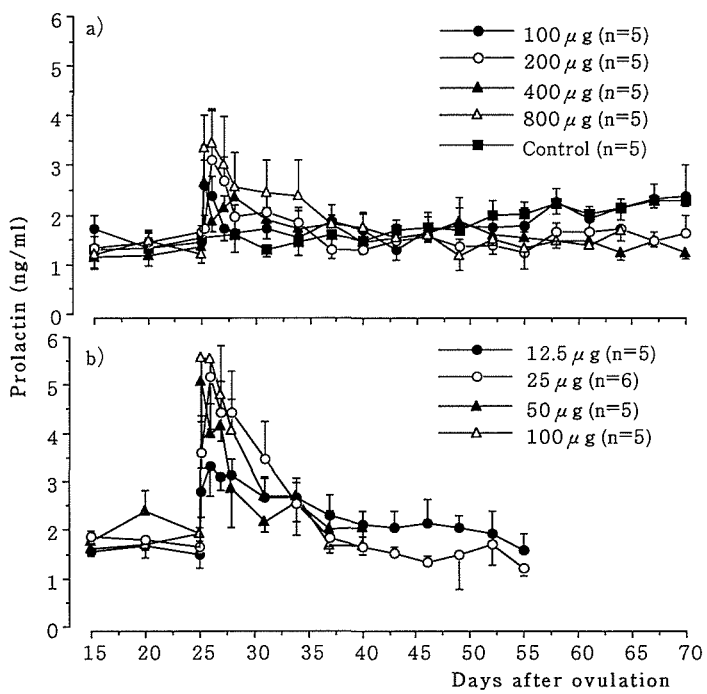

Fig. 2. Changes in the plasma PRL level (means ± SE) after PGA-E (a) and PGA-C (b) administration at 25 days after ovulation.

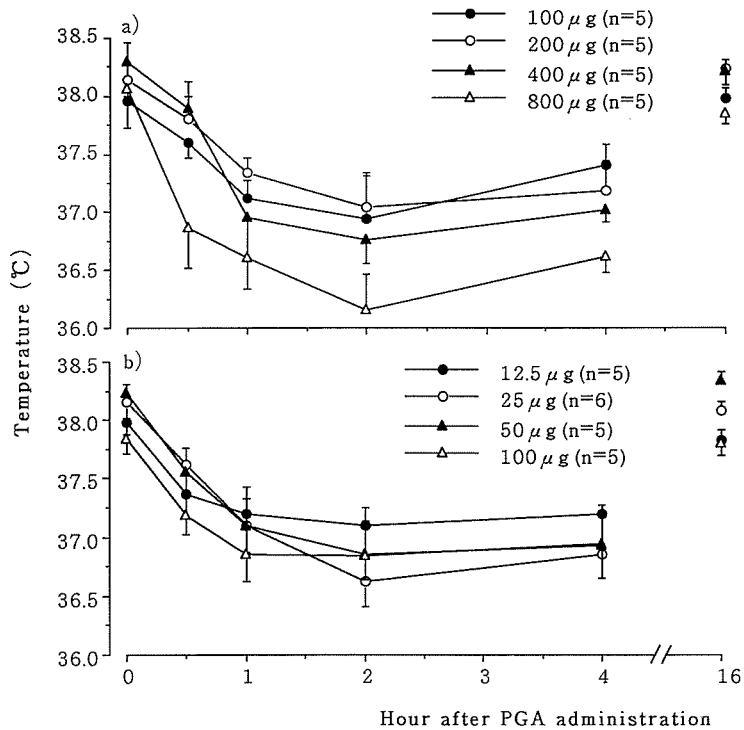

Fig. 3. Changes in body temperature (means ± SE) after PGA-E (a) and PGA-C (b) administration at 25 days after ovulation.

Table 1. Comparison of estrous cycle before and after PGA-E and PGA-C administration

PGA	Administra -tion dose (μg)	Number of bitches	Days from admin. to next estrus (Mean ± SE)	Estrous cycle (day: mean ± SE)		
				Pre-treatment* (A)	Post-treatment (B)	(A)-(B)
PGA-E	100	5	103.6 ± 25.3	176.0 ± 18.8	130.8 ± 27.2[a]	45.2 ± 13.4
	200	5	107.4 ± 25.2	207.4 ± 8.6	139.0 ± 27.4[a]	67.8 ± 19.6
	400	5	84.4 ± 13.7[c]	176.6 ± 12.9	116.6 ± 13.9[a,c]	60.0 ± 15.9
	800	5	76.6 ± 10.5[c]	194.2 ± 16.2	110.2 ± 9.9[b,c]	84.0 ± 7.8
PGA-C	12.5	5	65.0 ± 3.8[c]	162.0 ± 9.9	98.0 ± 3.3[b,c]	64.0 ± 8.8
	25	6	71.0 ± 16.3[c]	164.7 ± 10.7	102.1 ± 16.6[a,c]	67.7 ± 12.6
	50	5	76.0 ± 11.6[c]	161.8 ± 5.3	109.6 ± 11.9[b,c]	52.2 ± 12.7
	100	5	68.8 ± 11.8[c]	178.8 ± 12.4	102.0 ± 12.4[b,c]	77.2 ± 9.3
Control		6	160.4 ± 15.1	189.0 ± 10.8	185.0 ± 17.0	4.0 ± 13.5

*: Mean (± SE) days in the two estrus cycles before PGA administration.
Significantly different from pre-treatment at $p<0.05$ (a), $p<0.01$ (b).
Significantly different from Control at $p<0.01$ (c).

but all side effects were observed in the remaining dogs. These symptoms disappeared 3 hr after administration.

Influence of PGA administration on body temperature: The changes in body temperature after PGA administration are shown in Fig. 3; body temperature reached a minimum 2 hr after administration of PGA-E, and mean body temperature decreased to 36.2°C in the 800-μg group, suggesting that body temperature decreases in a dose-dependent manner. Furthermore, body temperature reached a minimum 2 hr after administration of PGA-C, and there was no correlation between the dose and the rate of decrease in body temperature. Four hr after administration, body temperature did not return to the pretreatment value in any dose group treated with PGA-E or PGA-C, but it returned to the pretreatment value in all dogs 16 hr after administration.

Recurrent estrus after PGA administration: The intervals between administration of PGAs or physiological saline and recurrent estrus are shown in Table 1. The interval between administration of PGA-E and recurrent estrus was shorter at a higher dose, but there were no significant differences

among the 4 dose groups. When comparing the interval with the mean estrus cycle at two points prior to administration in individual animals, the interval from administration of PGA-E until recurrent estrus was noticeably shortened ($p<0.01$, $p<0.05$), but there were no significant differences among the 4 dose groups.

There were no significant differences in the interval from administration of PGA-C until recurrent estrus among the 4 dose groups. When comparing the interval with the mean estrus cycle in individual animals, the interval was noticeably shortened ($p<0.01$, $p<0.05$), but there were no significant differences among the 4 dose groups.

There were no marked differences in the sexual cycle between the groups treated with 100 or 200 μg/kg of PGA-E and the control group, but there were marked differences between the groups treated with 400 or 800 μg/kg of PGA-E and the groups treated with PGA-C ($p<0.01$).

DISCUSSION

The single dose of PGA-E for promoting early regression of the functional corpus luteum in beagles was 800 μg, the highest dose used in our experiment. The plasma P_4 level returned to the baseline 8.6 ± 4.3 days after administration. This dose was approximately 1/2 and 1/12 of the clinical doses for pigs [24] and cattle [unpublished data Virbac, France, 1994], respectively. The dose should be increased to achieve luteal regression earlier. To achieve luteal regression earlier, the dose of PGA-E should be increased. With respect to administration of PGA-C, the plasma P_4 level returned to the baseline 2 days after administration at 50 μg/head. This dose was approximately 1/3 and 1/20 of the clinical doses for pigs and cattle, respectively. These results suggest that PGA-C is more useful than PGA-E for promoting luteal regression in dogs earlier by single-dose administration.

The side effects of PGA-E/PGA-C occurred in a dose-dependent manner, as demonstrated for luteal regression-promoting actions; in the high-dose groups, 3 hr were required until the symptoms disappeared. In particular, the body temperature in the group treated with 800 μg of PGA-E was more noticeably reduced to 36.2°C than values in the remaining dose groups and the PGA-C-treated groups. Thus, PGA-E may not be appropriate for promoting luteal regression in dogs in clinical application.

With respect to the responses of LH and PRL to administration of PGAs, there were no changes in the LH level after administration of PGA-E/PGA-C, but in the PGA-E- and PGA-C-treated groups, each of the PRL levels increased immediately after administration, reached a peak 4 hr or 1 day after administration, and then gradually decreased. The rate of increase in the PRL level in the PGA-C-treated dogs was greater than that in the PGA-E-treated dogs. The PRL level was higher at a higher dose of PGAs. In addition, the PRL level was higher when the P_4 level was more noticeably decreased after administration of PGAs. Therefore, a decrease in the plasma P_4 level related to administration of PGAs may have caused the positive feedback of pituitary hormones, promoting the secretion of PRL. This suggests that not LH but PRL is closely involved in stimulation of the corpus luteum 25 days after ovulation. This result supports the finding that PRL is a luteotrophic factor in dogs [3, 19, 21, 23].

PGA-E may be less useful than PGA-C for promoting luteal regression in dogs in clinical application.

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