Induction of Parturition in Bitches with Minimal Side Effects by Two Injections of a Low Dose of Fenprostalene, a Prostaglandin F_{2α} Analogue, and Pretreatment with Prifinium Bromide

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ABSTRACT. An experiment using 16 Beagle bitches (aged 11 months to 6 years and 2 months) in their 56th to 58th day of pregnancy was carried out to investigate the effects of two injections of a low dose of fenprostalene, a long-acting prostaglandin F_{2α} analogue, and pretreatment with prifinium bromide, a parasympathetic nerve blocking agent, on the induction of parturition and severity of side effects. The bitches were divided into three treatment groups: one injection of 5 µg/kg of fenprostalene (group I, n=5); one injection of 7.5 mg/head of prifinium bromide followed by one injection of 5 µg/kg of fenprostalene at 5 min after prifinium bromide injection (group II, n=6); and one injection of 7.5 mg/head of prifinium bromide followed by two injections of 2.5 µg/kg of fenprostalene, one injection at 5 min after prifinium bromide injection and the next at 1 hr after the fenprostalene first injection (group III, n=5). Following the injection of fenprostalene, side effects such as salivation, vomiting, colic symptoms, and watery diarrhea occurred most frequently (80–100% of cases) in group I bitches. Apart from colic symptoms, no side effects were observed in group III bitches. Group III bitches also showed the smallest increase in plasma cortisol concentration. No significant difference in the time to initiation of parturition was found between the three groups. The one-week survival rate of newborn puppies was highest in group III. The results showed that pretreatment with prifinium bromide and two injections of 2.5 µg/kg of fenprostalene can alleviate side effects following fenprostalene administration and have no adverse effect on the survival of newborn puppies, indicating that this method is a reliable and safe way of inducing parturition in bitches. —**KEY WORDS:** canine, fenprostalene, parturition induction, prifinium bromide, side effect.

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Prostaglandin F_{2α} (PGF_{2α}) or an analogous agent can be used for the induction of abortion [1, 2, 5, 11, 13, 18, 19, 21] or parturition [7, 8, 12, 23] and for the treatment of endometritis [16], pyometra [10, 16] or metritis [16, 22] in bitches. However, one clinical problem with the use of PGF_{2α} in bitches is that transient side effects (e.g., salivation, vomiting, colic symptoms, tachycardia, watery diarrhea) from the administration of an effective dose of PGF_{2α} or an analogous agent are stronger in bitches than in other animals.

We previously investigated the effect of the administration of fenprostalene, a long-acting PGF_{2α} analogue developed in USA, for the induction of abortion and parturition in bitches, and it was found that, although the duration of the above-mentioned side effects was slightly longer, the severity of the side effects was less than that caused by conventional PGF_{2α} treatment [7]. We have also reported that treatment with prifinium bromide, a parasympathetic nerve blocking agent that has a strong atropine action in bitches, before the administration of fenprostalene can induce parturition with a significant reduction in side effects [8]. However, a method that can completely eliminate side effects has not yet been found.

In this study, we investigated whether treatment with prifinium bromide followed by two divided injections of the minimal effective dose of fenprostalene (5 µg/kg) [8] can induce parturition while further reducing the side effects of fenprostalene.

MATERIALS AND METHODS

Animals: Sixteen pregnant Beagle bitches (aged 11 months to 6 years and 2 months) that were raised in our laboratory were used for the experiment. Treatment for the induction of parturition was carried out 56 to 58 days after the last mating or the last day of artificial insemination. No signs of parturition (nesting, lactation, etc.) were seen immediately before induction of parturition in any of the bitches. Moreover, the duration of gestation for cases of spontaneous delivery in a colony of Beagles identical to those used in this experiment was 61.4 ± 1.1 days (mean ± SD, n=7).

Method of parturition induction: The 16 pregnant bitches were divided randomly into three treatment groups. Bitches in group I (n=5) were injected subcutaneously with 5 µg/kg of fenprostalene (0.5 mg fenprostalene per 1 ml Synchrocept®, Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). Bitches in group II (n=6) were first injected intravenously with 7.5 mg/head of prifinium bromide (7.5 mg prifinium bromide per 1 ml Padrin®, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) followed 5 min later by subcutaneous injection of 5 µg/kg of fenprostalene. In group III (n=5), the bitches were first injected intravenously with 7.5 mg/head of prifinium bromide as in group II followed by two subcutaneous injections of fenprostalene, one injection of 2.5 µg/kg 5 min later and another injection of 2.5 µg/kg 1 hr after the fenprostalene

Table 1. Number of days of gestation at the time of fenprostalene administration in the Beagle bitches

Group I		Group II		Group III	
Dog No.	No. of days of gestation ^{a)}	Dog No.	No. of days of gestation	Dog No.	No. of days of gestation
1	58	6	57	12	56
2	58	7	57	13	58
3	56	8	56	14	58
4	58	9	58	15	57
5	57	10	57	16	57
		11	56		
57.4 ± 0.9 ^{b)}		56.8 ± 0.8		57.2 ± 0.8	

a) Number of days after the last mating or artificial insemination.

b) Mean ± SD.

first injection. There were no significant inter-group differences in the number of days of gestation (mean ± SD) at the time of treatment: 57.4 ± 0.9 days in group I vs 56.8 ± 0.8 days in group II vs 57.2 ± 0.8 days in group III (Table 1). All the bitches were fasted for 24 hr before the start of treatment.

Observation of parturition induction: Following the administration of fenprostalene, observations were made at 6-hr intervals until the appearance of signs of parturition and, after that, at 1-hr intervals until the initiation of parturition.

Observation of the general state of the dam: Body temperature, heart rate and respiratory rate were checked a total of 12–15 times at intervals of 15 min to 4 hr from just before the administration of fenprostalene until 22 hr after administration. Following that, observations were made at 6-hr intervals until the initiation of parturition. The occurrence and severity of side effects (salivation, vomiting, colic symptoms, and watery diarrhea) following fenprostalene administration were also checked until 10 hr intervals after administration. Each of the above four side effects was scored according to severity: 2 points for severe, 1 point for slight, and 0 points for absence of the side effect. These points were totalled for each bitch and expressed as the side effects score for that bitch.

Blood sampling: Three-ml blood samples were collected from the median antebrachial vein at the same time intervals as those above for general body monitoring. The blood was immediately transferred to a test tube containing heparin, and after mixing, the solution was centrifuged (1,700 × g at 15 min) and the resultant plasma was stored at - 20°C until measurement.

Measurement of steroid hormones: The concentrations of progesterone and cortisol in plasma were measured by the enzyme immunoassays of Nakao *et al.* [14, 15].

Calculation of the survival rate of newborn puppies: The survival rate was calculated as the ratio of the number of puppies that survived for one week after birth to the number of puppies born.

Statistical evaluation: Student's *t*-test and the chi-square test were used for statistical evaluation of measurements.

RESULTS

Parturition induction: The times (mean ± SD) from fenprostalene administration until initiation of parturition were 32 ± 11 hr (group I), 36 ± 7 hr (group II) and 34 ± 5 hr (group III). No significant differences between groups were found. There were also no significant inter-group differences in the litter size per bitch (mean ± SD): 5.6 ± 0.9 puppies in group I vs 5.7 ± 0.5 puppies in group II vs 5.6 ± 1.1 puppies in group III (Table 2).

Changes in body temperature, respiratory rate and heart rate: Mean body temperatures declined significantly ($p < 0.01$) from 38.0–38.3°C just before fenprostalene administration to 36.3–36.8°C at 2 hr after fenprostalene administration. Mean respiratory rates, which were 42–71 times/min before fenprostalene administration, peaked in groups I and II at 197 and 236 times/min, respectively, 30 min after fenprostalene administration ($p < 0.01$) and in group III at 179 times/min 1 hr after fenprostalene administration ($p < 0.01$). The respiratory rates returned to the preadministration levels 10–18 hr after fenprostalene administration. Heart rates increased transiently after fenprostalene administration, but the increase was not significant. Changes in body temperature, respiratory rate and heart rate were similar in all three groups, and no clear inter-group differences were found.

Table 2. Litter size in the Beagle bitches

Group I		Group II		Group III	
Dog No.	Litter size	Dog No.	Litter size	Dog No.	Litter size
1	5	6	6	12	6
2	7	7	6	13	7
3	5	8	6	14	4
4	6	9	5	15	5
5	5	10	6	16	6
		11	5		
5.6 ± 0.9 ^{a)}		5.7 ± 0.5		5.6 ± 1.1	

a) Mean ± SD.

Table 3. Incidence, time to onset and duration of side effects following the administration of fenprostalene to induce parturition in Beagle bitches

		Side effect			
		Salivation	Vomiting	Colic symptoms	Watery diarrhea
Group I ^{a)}	Incidence (%)	100.0 (5/5) ^{d)}	80.0 (4/5)	100.0 (5/5)	80.0 (4/5)
	Time to onset (min)	28 ± 19 ^{e)}	30 ± 21	26 ± 21	34 ± 38
	Time of duration (min)	92 ± 29	90 ± 21	128 ± 67	56 ± 33
Group II ^{b)}	Incidence (%)	16.7* (1/6)	33.3 (2/6)	66.7 (4/6)	0* (0/6)
	Time to onset (min)	60	88 ± 61	40 ± 12	–
	Time of duration (min)	60	2 ± 0**	37 ± 36*	–
Group III ^{c)}	Incidence (%)	0** (0/5)	0* (0/5)	40.0 (2/5)	0* (0/5)
	Time to onset (min)	–	–	67 ± 33*	–
	Time of duration (min)	–	–	50 ± 6	–

a) One subcutaneous injection with 5 µg/kg of fenprostalene.

b) Intravenous injection with 7.5 mg/head of profininium bromide followed by one subcutaneous injection with 5 µg/kg of fenprostalene.

c) Intravenous injection with 7.5 mg/head of profininium bromide followed by two subcutaneous injections with 2.5 µg/kg of fenprostalene.

d) Number of bitches in which side effects appeared/number of treated bitches.

e) Mean ± SD.

* p<0.05, ** p<0.01 when compared with values of group I.

Side effects in the digestive system: The incidence, time to onset and duration of side effects in each group following fenprostalene administration are shown in Table 3, and the scores of side effects in each group are shown in Table 4. Side effects in the digestive system (salivation, vomiting, colic symptoms, and watery diarrhea) occurred more frequently in group I than in group II. The only side effect that was seen in group III was colic symptoms, which occurred in only two of the five (40.0%) bitches. The time to onset of colic symptoms was also significantly longer (p<0.05) in group III than in group I. A significant reduction (p<0.05–0.01) in the duration of vomiting and colic symptoms was also seen in group II compared to group I. A comparison of the severities of side effects on the digestive system, based on the side effect scores, showed that there was a significant reduction in the severity of side effects in group III compared to those in group I (p<0.001) and group II (p<0.05).

Changes in plasma progesterone concentration: The plasma progesterone concentration immediately before fenprostalene administration was high in all bitches (6.4–17.0 ng/ml) (Table 5). Changes in the plasma progesterone concentration in each group are shown in Table 6. Plasma progesterone concentrations declined from 10.2–11.4 ng/ml just before fenprostalene administration to 4.1–5.4 ng/ml at 1 hr after administration and then declined rapidly to 1.2–2.6 ng/ml at 8 hr after administration (p<0.05–0.001), leading to parturition. No significant differences in the pattern of changes in plasma progesterone concentration were found between the three groups.

Changes in plasma cortisol concentration: Changes in the plasma cortisol concentration in each group are shown in Table 7. Plasma cortisol concentrations in all groups

Table 4. Side effects score for each Beagle bitch following injection of fenprostalene

Group I		Group II		Group III	
Dog No.	Score ^{a)}	Dog No.	Score	Dog No.	Score
1	8	6	2	12	1
2	4	7	1	13	0
3	4	8	1	14	1
4	7	9	2	15	0
5	4	10	1	16	0
		11	1		
5.4 ± 1.9 ^{b),***}		1.3 ± 0.5*		0.4 ± 0.5	

a) Each of the side effects (salivation, vomiting, colic symptoms and watery diarrhea) was scored according to severity: 2 points for severe, 1 point for slight, and 0 points for absence of the side effect. The side effects score is the total of these points for each bitch.

b) Mean ± SD.

* p<0.05, *** p<0.001 when compared with value of group III.

increased significantly and reached peaks at 2–4 hr after fenprostalene administration (p<0.01–0.001), and then the concentrations returned to the preadministration levels at 22 hr after administration. Peak plasma cortisol concentrations were significantly lower (p<0.05) in groups II and III than in group I, and lower (tendency) in group III than in group II.

Survival rates of newborn puppies: One-week survival rates of newborn puppies were 67.9% (group I), 82.3% (group II) and 97.1% (group III). Survival rates were significantly higher (p<0.05–0.01) in group III than in groups I and II.

Table 5. Plasma progesterone concentration immediately before fenprostalene administration in the Beagle bitches

Group I		Group II		Group III	
Dog No.	Plasma progesterone level (ng/ml)	Dog No.	Plasma progesterone level (ng/ml)	Dog No.	Plasma progesterone level (ng/ml)
1	15.6	6	17.0	12	9.6
2	8.0	7	8.6	13	11.0
3	8.0	8	13.4	14	9.4
4	6.4	9	7.4	15	9.8
5	13.0	10	8.1	16	17.0
		11	9.0		
10.2 ± 3.9 ^{a)}		10.6 ± 3.8		11.4 ± 3.2	

a) Mean ± SD.

Table 6. Changes in the plasma progesterone concentration after fenprostalene administration in each group of Beagle bitches

Elapsed time after fenprostalene administration ^{a)} (hr)	Plasma progesterone concentration (ng/ml)		
	Group I (n=5)	Group II (n=6)	Group III (n=5)
0	10.2 ± 2.0 ^{b)}	10.6 ± 1.4	11.4 ± 1.6
0.5	6.3 ± 1.3	3.8 ± 0.4	5.5 ± 0.9
1	5.4 ± 1.2	4.1 ± 0.5	4.8 ± 1.0
2	5.1 ± 1.0	3.1 ± 0.3	2.5 ± 0.7
4	3.5 ± 0.6	2.3 ± 0.5	1.6 ± 0.4
8	2.6 ± 0.7	1.2 ± 0.3	1.3 ± 0.3

a) Elapsed time after initial fenprostalene injection in group III.

b) Mean ± SE.

Table 7. Changes in the plasma cortisol concentration after fenprostalene administration in each group of Beagle bitches

Elapsed time after fenprostalene administration ^{a)} (hr)	Plasma cortisol concentration (ng/ml)		
	Group I (n=5)	Group II (n=6)	Group III (n=5)
0	28.3 ± 8.2 ^{b)}	20.3 ± 2.0	18.4 ± 5.7
0.5	47.2 ± 8.6	83.7 ± 12.0	60.0 ± 9.5
1	111.4 ± 9.2	79.7 ± 2.6	69.6 ± 9.5
2	130.0 ± 12.9	82.8 ± 16.3	80.8 ± 12.0*
4	110.0 ± 12.8	90.7 ± 9.6*	69.2 ± 10.4
8	70.6 ± 10.4	50.7 ± 11.7	54.6 ± 9.9
14	71.6 ± 9.2	50.7 ± 10.9	62.8 ± 14.8
18	36.5 ± 12.8	53.0 ± 16.4	28.8 ± 5.3
22	26.3 ± 6.8	29.2 ± 5.6	32.2 ± 11.7

a) Elapsed time after initial fenprostalene injection in group III.

b) Mean ± SE.

* $p < 0.05$ when compared with peak value of group I.

DISCUSSION

Pretreatment with prifinium bromide and two injections of a low dose of fenprostalene both clearly reduced the severity of salivation, vomiting, colic symptoms, and watery diarrhea following fenprostalene administration. The occurrence of these side effects following injection of PGF_{2α} may be caused by direct action of PGF_{2α} on the digestive system through bonding to receptors in the gastrointestinal smooth muscle [3, 9] or by indirect action on the digestive system via parasympathetic nerves [3]. The authors previously reported that salivation and diarrhea were completely suppressed by pretreatment with prifinium bromide, a parasympathetic nerve blocking agent, suggesting that both of these side effects are caused by the action of PGF_{2α} via parasympathetic nerves [8]. Lein *et al.* [13] reported that 50 μg/kg of atropine injected intramuscularly at the same time as PGF_{2α} administration reduced the severity of side effects such as salivation. However, prifinium bromide has a stronger effect than atropine, especially on the digestive system [6], and this is thought to have been the reason for the more extensive and longer inhibition of side effects in the digestive system in the present study.

Also, as side effects of PGF_{2α} are dose-dependent [3,

24], the authors investigated in a previous study [8] the effect of the dose of fenprostalene on side effects and found that injection of 5 μg/kg (minimum effective dose) of fenprostalene reduced the occurrence of side effects while producing similar parturition induction results to those obtained by the conventional dose of 20 μg/kg of fenprostalene. As it has been shown that the *in vivo* half-life of prifinium bromide is 2 hr [17], the half-life of fenprostalene is 18–23 hr, and side effects after fenprostalene administration occur within one hr [7, 8], we decided to test the effect of two injections of 2.5 μg/kg of fenprostalene after prifinium bromide administration. Compared to one injection of 5 μg/kg of fenprostalene, two injections of 2.5 μg/kg of fenprostalene reduced, or completely inhibited in some cases, the severity of side effects following fenprostalene administration.

The mean time from the administration of fenprostalene until the initiation of parturition in the present study is almost the same as the time of 31.4 ± 5.7 hr reported in a study using one injection of 20 μg/kg of cloprostenol [12], confirming that the method used in the present study (i.e., pretreatment with prifinium bromide and two injections of

fenprostalene) does not have an adverse effect on the induction of parturition.

Moreover, neither the inclusion of prifinium bromide pretreatment nor the division of fenprostalene injections lowered the survival rate of newborn puppies. Rowlands [20] reported that the general survival rate in the first week after birth, in which the mortality rate is highest, is 72.1%. The survival rates in the present study were similar to, and in some cases higher than, this rate, indicating that injection of prifinium bromide has no adverse effect on the survival of newborn puppies.

The fact that the plasma progesterone concentration immediately before fenprostalene administration was high in all bitches confirms that corpora lutea regression had not started in any of the bitches at the time of fenprostalene administration. The fact that no significant difference in the fluctuation pattern of plasma progesterone concentration was found between the three groups confirms that the corpora lutea sufficiently regresses even with prifinium bromide pretreatment or two injections of a low dose of fenprostalene. Moreover, although the number of days of gestation at the time of treatment ranged from 56 to 58 days, the fact that the plasma progesterone concentration rapidly decreased after fenprostalene administration and parturition was induced within 48 hr in all of the bitches suggests that there were no differences in the drug sensitivity of the target organ of fenprostalene (i.e., the corpora lutea).

Although the plasma cortisol concentration increased rapidly after fenprostalene administration, this rapid increase was thought to be due to physiological stresses caused by the side effects of fenprostalene [4]. The fact that the peaks of plasma cortisol concentration following fenprostalene administration were in the order of group I > group II > group III suggests that stress is suppressed to some degree by pretreatment with prifinium bromide and divided injections of fenprostalene.

The results of this study showed that when using fenprostalene to induce parturition in bitches, side effects can be significantly alleviated by pretreatment with prifinium bromide, a parasympathetic nerve blocking agent, and two injections of a low dose of fenprostalene. As pretreatment with prifinium bromide and divided injections of a low dose of fenprostalene showed no adverse effects on either parturition induction or the survival rate of newborn puppies, this method is considered to be a reliable and safe way of inducing parturition in bitches.

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