

Fenprostalene-induced Abortion in Bitches

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ABSTRACT. A long-acting prostaglandin $F_{2\alpha}$ analogue, fenprostalene, was subcutaneously administered singly at doses of 5, 10, 25, 50, 100 or 150 μg on day 25 after ovulation during pregnancy in bitches ($n=4$ for each dosage), and maintenance of pregnancy, changes in plasma progesterone concentration, interval between the treatment and subsequent estrus and conception rate at that estrus were studied. Abortion was associated with a decrease in the plasma progesterone concentration below about 2 ng/ml , and the abortifacient effect was dose-dependent. Administration of 50 μg or more of fenprostalene induced abortion in all dogs 3 to 13 days after the treatment. The interval between administration and subsequent estrus was markedly shorter in the aborted bitches than in the non-aborted ones ($P<0.01$). The conception rate at the estrus in the aborted dogs was 50%, whereas all of the bitches who had not aborted became pregnant. The results indicate that a single administration of fenprostalene could induce abortion during mid-pregnancy in bitches, and the subsequent estrus may come early with a low conception rate.

KEY WORDS: abortion, canine, fenprostalene, progesterone, recurrence of estrus.

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In dogs, ovariectomy during pregnancy immediately decreases peripheral blood progesterone (P_4) levels to the baseline, causing abortion [15]. Thus, to maintain pregnancy in dogs, the corpus luteum is required during pregnancy. To promote luteal regression in dogs, natural prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$) [1, 2, 12, 13, 17, 19] and $\text{PGF}_{2\alpha}$ analogue [3, 14, 16, 18] are applied in clinical practice. In particular, it is known that $\text{PGF}_{2\alpha}$ analogue is more effective than natural $\text{PGF}_{2\alpha}$ for inducing abortion in dogs. Fieni *et al.* [3] subcutaneously administered a $\text{PGF}_{2\alpha}$ analogue, cloprostenol (2.5 $\mu\text{g/kg}$), to dogs on days 29 to 55 of pregnancy 3 times at 48 hr intervals, and reported that abortion was induced in 53 (79.1%) of 67 dogs. However, using their administration method, the number of administrations is high, and the rate at which abortion is induced may not be satisfactory. Thus, currently, there is no safe and accurate method for inducing abortion in dogs.

We previously investigated the influence of fenprostalene (BOVILENE® Syntex Animal Health, Inc.), which is a $\text{PGF}_{2\alpha}$ analogue with prolonged activity, on estrus/luteal functions in beagles [5]. Administration of 50 μg or more of fenprostalene 25 days after ovulation (during the luteal phase) decreased peripheral blood P_4 levels to the baseline (approximately 1 ng/ml) 2 days after administration regardless of the dose, and early luteal regression was achieved. Furthermore, Iseki *et al.* [6] and Moriyoshi *et al.* [7] reported that fenprostalene could be applied for the induction of parturition. However, none of the previous studies reported the induction of abortion in mid-pregnancy.

In this study, we determined the dose of fenprostalene required for inducing abortion in beagles. We also investigated fertility related to estrus after abortion. Administration was performed 25 days after ovulation, when the corpus luteum functions most markedly during pregnancy in dogs.

MATERIALS AND METHODS

Animals: The dogs used were 29 female beagles 1–7 years of age bred successively at the Nippon Veterinary and Animal Science University. Twenty-four animals were allocated to a fenprostalene treatment group and five were allocated to an intact group. Of the 24 animals in the fenprostalene treatment group, 11 were used during the initial estrus. The remaining 10 and three animals were multiparous and nulliparous, respectively, and their estrus cycles were normal. Eight male beagles 1–7 years of age were used for mating. The experimental dogs were maintained in cages measuring 160 \times 75 \times 65 cm and were fed commercial dog food (Hill's Canine Maintenance, U.S.A.) once daily. Water was supplied three times daily: early in the morning, at noon, and in the evening.

Timing the onset of ovulation and mating: Estrus in the experimental dogs was diagnosed by observing the leakage of bloody mucus from the vulva and swelling of the vulva. Peripheral blood was collected from the dogs presenting signs of estrus once daily from the 6th day after the onset of estrous bleeding, as we previously reported, and the onset of ovulation was timed from peripheral blood P_4 levels [4]; the first day that a peripheral P_4 level of 2 ng/ml or over was recorded was regarded as the day of ovulation. The female dogs were mated once with male dogs during the 3-day period after ovulation that is suitable for mating, and were observed for conception. Pregnancy was diagnosed with ultrasonic diagnostic equipment (ECHOVISION SSD-500EV, Aloka Co., Japan) before fenprostalene administration at 25 days after ovulation.

Fenprostalene administration: Fenprostalene (500 μg fenprostalene per 1 ml, BOVILENE® Syntex Animal Health, Inc., U.S.A.) was administered at 25 days after ovulation. Groups of 4 dogs were administered 150, 100, 50,

25, 10 and 5 $\mu\text{g}/\text{head}$ of fenprostalene, respectively. The volume of dosage per head was adjusted to 0.5 ml by diluting fenprostalene with a diluent (Macrogol 400, Yoshida Pharmaceutical Co., Ltd.).

Measurement of plasma progesterone levels: To follow changes in blood P_4 levels in the experimental dogs after fenprostalene administration, blood was collected from the cephalic vein at 25 days after ovulation before fenprostalene administration and at 1, 2, 4, 8 and 12 hr after administration, and then every day for the following 5 days, and thereafter at 3-day intervals until blood P_4 levels dropped below 1 ng/ml or up to 3 days after parturition. If the female dog aborted, blood was collected every day for 3 days from the day of abortion. To follow changes in blood P_4 levels in the untreated dogs, blood was collected at 3-day intervals from 24 or 60 days after ovulation, or up to parturition, and then every day for the following 3 days. Immediately after blood collection, plasma was separated by centrifugation in a refrigerated centrifuge and stored at -30°C until measurement of P_4 levels. The blood P_4 level was measured by an enzyme immunoassay method as previously reported [8, 16].

Observation of the side effects after fenprostalene administration: After fenprostalene administration, dogs were observed for side effects, such as hypersalivation, vomiting, diarrhea, and hypothermia, until blood collection at 12 hr after administration. Temperature was measured rectally.

Abortion after administration of fenprostalene: The presence or absence of aborted fetuses was examined everyday (morning, noon, evening; 3 times a day) after administration. Furthermore, the vulva was investigated to examine for the presence or absence of leakage related to aborted fetuses. Using an ultrasonograph, we examined the presence or absence of fetuses and whether they were alive or dead everyday after fenprostalene administration. In dogs in which fetuses were alive until 40 days after ovulation, findings were investigated every 5 days after abortion.

Recurrence of estrus after fenprostalene administration and breeding test: Seventeen of the 24 fenprostalene-administered dogs were observed for the recurrence of estrus, and the estrus cycle was compared with that in untreated pregnant bitches. These dogs were mated once with male dogs during the 3- to 4-day period after ovulation that is suitable for mating, and were observed for conception.

Statistical analysis: The results obtained in this study were analyzed by Student's *t*-test.

RESULTS

Induction of abortion by fenprostalene administration: The interval between fenprostalene administration at various doses and abortion is shown in Table 1. Briefly, abortion was induced in all 12 dogs treated with 50 to 150 μg of fenprostalene. In these dogs, the interval between administration and abortion ranged from 3 to 13 days regardless of the dose of fenprostalene, with a mean of 7.8 ± 1.0 (SE) days. In the 25 μg and 10 μg groups, abortion occurred 8 to

15 days after administration in 3 of 4 dogs and in 2 of 4 dogs, respectively. However, the remaining 3 dogs delivered normally 61 to 65 days after mating. In the 5 μg group, pregnancy was maintained in all 4 dogs. These dogs normally delivered 5 to 9 newborns 60 to 63 days after mating (35 to 40 days after administration).

There was no significant difference in the pregnancy period and number of newborns between the 7 dogs in which pregnancy was maintained despite fenprostalene administration and the 5 untreated pregnant dogs.

Time course of changes in peripheral blood progesterone levels after fenprostalene administration: Figure 1 shows the time course of changes in peripheral blood P_4 levels (mean \pm SE) after the administration of 150, 100, 50, 25, 10, 5 $\mu\text{g}/\text{head}$ of fenprostalene to 6 groups of pregnant dogs at 25 days after ovulation. They showed similar time-dependent changes in peripheral blood P_4 levels after fenprostalene administration, with no significant differences among the 3 groups administered 150, 100, 50 $\mu\text{g}/\text{head}$ of fenprostalene: the peripheral blood P_4 levels showed baseline levels around 1 $\mu\text{g}/\text{ml}$ from 2 days after administration. Intervals after the peripheral blood P_4 level decreased to 2.0 ng/ml or less until abortion ranged from 3 to 13 days.

In 3 of 4 dogs treated with 25 μg of fenprostalene, abortion was induced, and the intervals after the peripheral blood P_4 level decreased to 2.0 ng/ml or less until abortion ranging from 4 to 10 days post administration. However, in 1 dog in which pregnancy was maintained (Bitch No. 179), the peripheral blood P_4 level transiently decreased, but was then approximately maintained at 2.0 ng/ml until delivery.

In 2 of 4 dogs treated with 10 μg of fenprostalene, abortion was induced, and the intervals after the peripheral blood P_4 level decreased to 2.0 ng/ml or less until abortion were 6 and 10 days, respectively. However, in the 2 dogs that delivered, peripheral blood P_4 levels transiently decreased to approximately 2 ng/ml, which was maintained until delivery.

In 4 dogs treated with 5 μg of fenprostalene, the change was transient, and complete luteal regression did not occur.

Side effects of fenprostalene administration: With respect to side effects related to fenprostalene administration, salivation, vomiting and diarrhea were observed 1 hr or more after administration in dogs treated with 50 to 150 μg of fenprostalene. These symptoms disappeared 4 to 8 hr after onset. Furthermore, body temperature fell until 4 hr after administration, but rose 8 hr or more after administration. In 3 of the 5 dogs treated with 10 to 25 μg of fenprostalene abortion was induced, and vomiting and salivation were observed, but these symptoms were milder than those in the high dose group. Furthermore, in the remaining 2 dogs, there were no side effects. In the 3 dogs treated with 10 to 25 μg of fenprostalene in which pregnancy was maintained and 4 dogs treated with 5 μg of fenprostalene, vomiting or salivation was observed 1 hr and 2 hr after administration in only 2. In the remaining dogs, no side effects developed.

Recurrence of estrus after fenprostalene administration and conception rate at that estrus: The time periods

Table 1. Induction of abortion by fenprostalene administration

Administration dose (μg)	Bitch No.	Days from admin. to abortion	Gestation period (No. of pups)	Abortion rates
150	185	4	— ^{a)}	
	190	7	—	4/4
	245	6	—	100%
	254	12	—	
100	217	12	—	
	231	8	—	4/4
	253	3	—	100%
	257	5	—	
50	139	13	—	
	200	8	—	4/4
	224	6	—	100%
	243	10	—	
25	179	parturition	65 (2)	
	236	15	—	3/4
	247	8	—	75%
	251	9	—	
10	235	13	—	
	252	parturition	61 (6)	2/4
	255	parturition	62 (9)	50%
	260	13	—	
5	258	parturition	62 (9)	
	263	parturition	63 (5)	0/4
	264	parturition	60 (5)	0%
	273	parturition	61 (5)	
Mean		8.9	62.0 (5.9)	
\pm SE		0.9	0.7 (1.0)	

a): abortion

between fenprostalene administration and subsequent estrus are shown in Table 2. In the 12 bitches which aborted, the time periods between administration and the onset of estrus were a mean of 76.6 ± 15.4 days, and a mean of 178.2 ± 26.0 days in the 5 bitches which delivered. In 12 dogs in which abortion was induced, the estrous cycle was markedly shortened ($P < 0.01$) when compared with untreated pregnant dogs, whereas in dogs in which pregnancy was maintained, there was no significant difference between the estrous cycle.

During the subsequent estrus after fenprostalene administration, 11 of the 17 bitches tested became pregnant, the overall conception rate being 60.0%: the conception rates for the 12 bitches which aborted after treatment and the 5 bitches which delivered were 6/12 (50.0%) and 5/5 (100%), respectively. The next estrus was observed 139.4 ± 18.1 days after fenprostalene administration in 11 fertilized dogs and 46.1 ± 15.8 days in six non-fertilized dogs, showing that the period was significantly shortened ($P < 0.01$).

DISCUSSION

This study showed that a single subcutaneous administra-

tion of $50 \mu\text{g}/\text{head}$ or more of fenprostalene could induce abortion 25 days after ovulation in pregnant bitches. However, the interval between fenprostalene administration and abortion ranged from 3 to 13 days regardless of the dose. It was previously reported that $\text{PGF}_{2\alpha}$ -1052 on days 25 to 45 of pregnancy induced abortion 3 or 4 days after administration [16]. In this study, peripheral blood P_4 level decreased to 1.0 ng/ml or less 2 days after fenprostalene administration. Though it differs slightly between researchers, the peripheral blood P_4 level at abortion was $0.6\text{--}1.4 \text{ ng/ml}$ [1], $1.3 \pm 0.1 \text{ ng/ml}$ or less [12], and 1.0 ng/ml or less [19]. Furthermore, another report indicated that abortion occurred within 24 hr after peripheral blood P_4 levels decreased to 2.0 ng/ml or less. However, in this study, abortion took time to occur after the peripheral blood P_4 level decreased to the baseline. Therefore, it may be difficult to predict the day of abortion based on peripheral blood P_4 levels alone. This may be a feature of fenprostalene, but the reason is unclear. The inability to predict the days required to cause abortion may be problematic in the clinical application of fenprostalene. In the future, the mechanism should be clarified.

In this study, the fertility rate related to estrus after fenprostalene administration was low (50.0%) in the 12 dogs

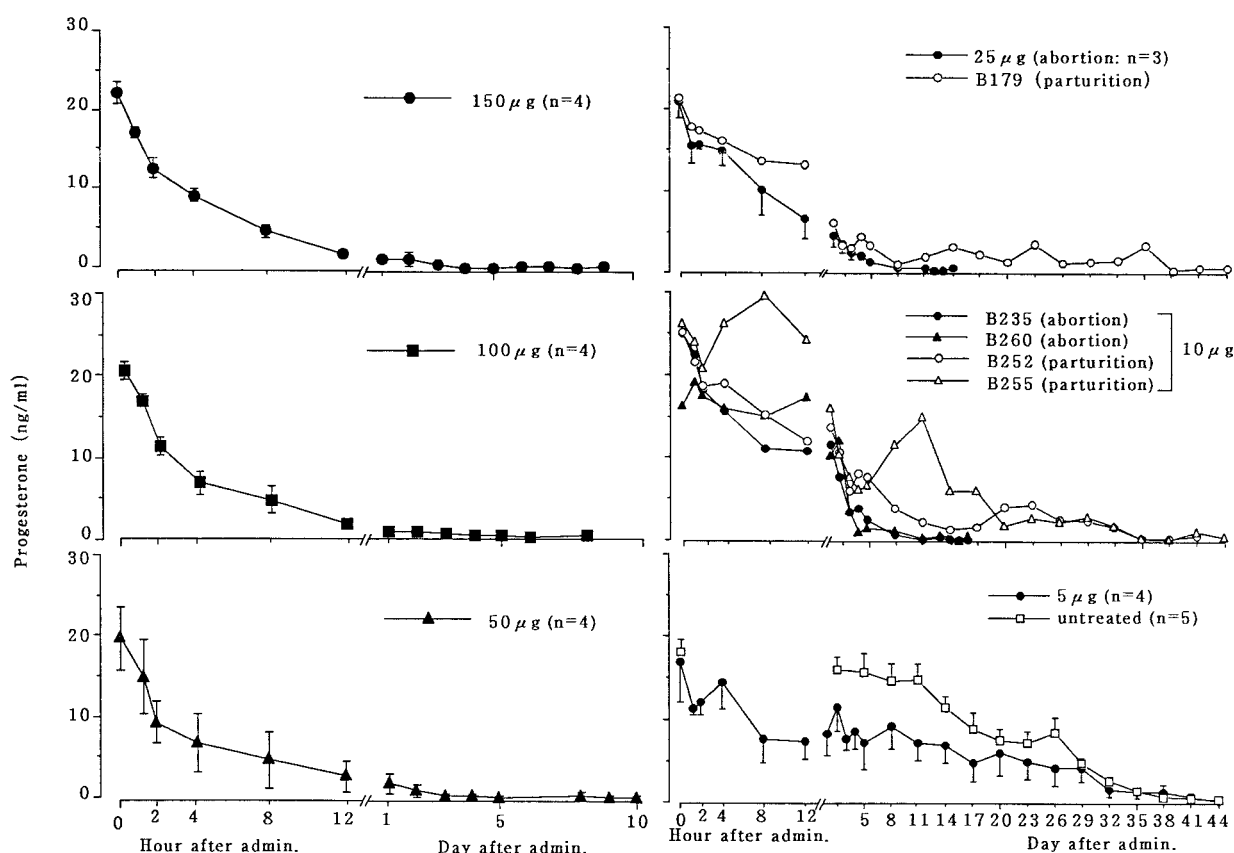


Fig. 1. Time course of changes in the peripheral blood P₄ levels after the subcutaneous administration of doses of 5–150 µg/head of fenprostalene to pregnant bitches at 25 days after ovulation.

which aborted. However, in the 5 dogs in which pregnancy was maintained, the fertility rate was 100%. The duration of the period after fenprostalene administration to the next estrus in the non-fertilized dogs was significantly shortened to 46.2 ± 15.8 days. In our previous study, among bitches treated with fenprostalene during the luteal phase, dogs with estrus about 2 months or more after administration became pregnant [5]. However, among dogs treated with fenprostalene during pregnancy, some bitches with estrus 80 or 98 days after administration were infertile. This may have been because a long period was required for uterine repair after abortion.

Side effects appeared in bitches in which abortion was induced after administration of fenprostalene, but they were not severe. Furthermore, recent studies have investigated a method in which side effects related to PGF_{2α} administration are relieved and abortion is induced by concurrently administering PGF_{2α} and anti-prolactins such as bromocriptine [9] and cabergoline [9–11]. In the future, combination therapy with fenprostalene and these agents should be investigated.

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Table 2. Interval between fenprostalene administration and subsequent estrus and conception rates

Administration dose (μg)	Bitch Number	Days from administration to next estrus		Result of conception (Number of pups)	Conception rates
		A ^{a)}	B ^{b)}		
150	185		22	— ^{c)}	
	245		58	+ (1) ^{d)}	1/3 (33.3%)
	254		17	—	
100	217		45	—	
	231		57	+ (5)	1/3 (33.3%)
	253		15	—	
50	139		96	+ (abortion)	
	243		175	+ (8)	2/2 (100%)
25	236		98	—	
	251		149	+ (6)	1/2 (50.0%)
10	235		80	—	
	252	268		+ (4)	
	255	172		+ (7)	3/4 (75.0%)
	260		107	+ (6)	
5	258	142		+ (6)	
	263	166		+ (1)	3/3 (100%)
	264	143		+ (3)	
Mean		178.2 ^{a)}	76.6 ^{b)}	(4.7)	11/17
$\pm\text{SE}$		26.0	15.4	(0.8)	(64.7%)
untreated	222	226		+ (3)	
	239	139		+ (7)	
	273	119		+ (2)	
	282	133		+ (5)	
	297	161		+ (6)	
Mean		155.6 ^{e)}		(4.6)	
$\pm\text{SE}$		21.1		(1.0)	

a): maintained pregnancy after fenprostalene administration.

b): abortion after fenprostalene administration.

c): day (mean \pm SE) from fenprostalene administration to next estrus in 6 sterile bitches; 46.1 ± 15.8 .d): day (mean \pm SE) from fenprostalene administration to next estrus in 11 pregnant bitches; 139.4 ± 18.1 .

e): day from 25 days after ovulation to next estrus.

Significantly different between a) and b), b) and e), c) and d), c) and e) ($P < 0.01$).

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