NOTE *Theriogenology*

Changes in Prostaglandin E_2 Levels in Seminal Plasma during Ejaculation and the Effect of Exogenous Prostaglandin E_2 on Semen Volume in the Dog

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ABSTRACT. In healthy male dogs, peripheral plasma testosterone (T), prostaglandin E_2 (PGE_2) and seminal plasma PGE_2 levels were measured before, during and after ejaculation, and semen quality was examined after oral administration of PGE_2. Plasma T and PGE_2 levels did not change during these periods, but the seminal plasma PGE_2 level of combined the first and second fractions was significantly higher than those at 0–5 and 5–10 min after the start of ejaculation of the third fraction. Semen volume but not quality increased after PGE_2 administration. In conclusion, large amounts of PGE_2 are released from the prostate gland during the early part of ejaculation, and PGE_2 plays an essential role in secretion of seminal plasma.

KEY WORDS: canine, ejaculation, prostaglandin E_2, seminal plasma.

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Prostaglandins (PGs) are eicosanoid hormones. Many different PGs are contained within ejaculated semen [18, 19] and are mainly synthesized in the accessory reproductive glands, such as the seminal vesicles and prostate gland [2]. It was previously shown that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administration increased the total number of sperm or sperm concentration in many mammals including rabbits and bulls [6, 14], but did not change the number of spermatozoa in dogs [20]. On the other hand, prostaglandin E_2 (PGE_2) administration in rabbits led to an increase in sperm concentration [14]. While $PGF_{2\alpha}$ supplementation suppressed sperm motility and induced sperm membrane damage and permeability in bulls [5], it was shown that PGE_2 could improve the motility of spermatozoa in men [4]. Moreover, PGE induced both Ca^{2+} influx through the PGE receptor and the acrosome reaction in human spermatozoa [16]. However, to our knowledge, the kinetics and function of PGE_2 during ejaculation in dogs remain unclear.

The primary purpose of this study was to evaluate the association between seminal emission and PGE_2 secretion by measurement of PGE_2 in peripheral blood and seminal plasma in dogs. Moreover, semen quality was examined for investigation of PGE_2 function by PGE_2 administration.

After a set of indwelling needles was inserted into the cephalic vein of 5 male dogs (2–4 years old) with normal semen quality, blood samples were collected 10 and 5 min before ejaculation; immediately before ejaculation; 5, 10 and 15 min after the start of ejaculation; and 0, 5 and 10 min after the end of ejaculation. The semen was collected by digital manipulation. The blood samples were used to measure testosterone (T) and PGE_2 levels. All T measurements were obtained with an enzyme-linked fluorescence assay kit (VIDAS testosterone, SYSMEX bioMerieux, Tokyo, Japan) using an automated fluorescence immunochemistry analyzer (SPOTCHEM VIDAS SV-5010; ARKRAY, Kyoto, Japan). PGE_2 concentration was measured using a prostaglandin E metabolite enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, U.S.A.) and a fluorescence microplate reader (Powerscan HT; DS Pharma Biomedical, Osaka, Japan). The amount of PGE2 in seminal plasma was calculated by the following formula: PGE2 concentration (*p*g/m*l*) in each fraction × seminal plasma volume (m*l*) of each fraction. This value was expressed as the mean ± standard error (SE). Time course changes in plasma T and PGE_2 levels were evaluated using a one-way analysis of variance (one-way ANOVA) to determine differences among the groups. When a significant difference was found in one-way ANOVA, intergroup comparisons were undertaken using Tukey-Kramer's post hoc test and were considered statistically significant at P values less than 0.05.

Semen samples collected from the dogs were separated into the combined first and second fraction and the third fraction, which was ejaculated following the second fraction [7, 9]. To obtain separate fractions of ejaculated semen, the collection tubes were changed after collecting the end of the second fraction. In addition, the third fraction was collected every 5 min, separated and stored. PGE_2 levels were measured in seminal plasma samples extracted by centrifugation.

Furthermore, 4 of 5 male dogs used in the above experiment were given oral PGE_2 0.1 mg/kg (Dinoprostone; Kaken Pharmaceutical Co., Ltd., Tokyo, Japan), and semen collection was performed 1 hr after administration. Semen qualities including volume of each semen fraction and sperm number, motility, viability and abnormality were analyzed, and the effect of PGE_2 on ejaculation was examined by comparison of these variables between samples from dogs before PGE_2 administration (average values obtained from

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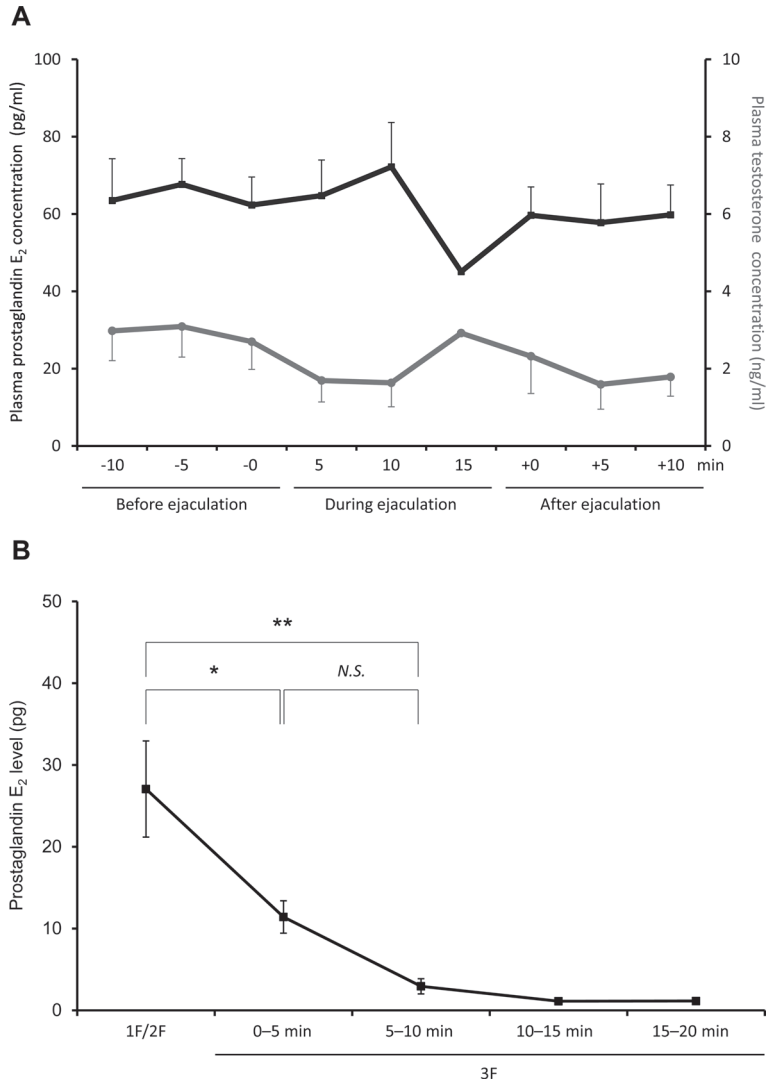

Fig. 1. (A) Time course changes in prostaglandin E_2 level (black line, mean ± standard error (SE), *p*g/m*l*) and testosterone level (gray line, mean ± standard error (SE), *n*g/m*l*) before and after the start of ejaculation in plasma of 5 normal dogs (n=5). (B) Time course changes in PGE_2 levels (mean ± SE, *p*g) in seminal plasma of these dogs. Ejaculated semen was divided into the combined first and second fractions (1F/2F) and 0–5, 5–10, 10–15 and 15–20 min after the start of ejaculation of the third fraction (3F) ejaculated following the second fraction, and PGE_2 levels were measured (n=5). *N.S.*, not significant. **P*<0.05; ***P*<0.01, for a 2–group comparison.

5 examinations of semen properties within 5 months, n=4) and samples from dogs after PGE_2 administration (the same dogs, n=4). In addition, blood was collected before and 1 hr after PGE_2 administration to confirm an increase in PGE_2 level. These variables were evaluated using a paired *t*-test and were considered statistically significant at *P* values less than 0.05.

Time course measurement of PGE_2 and T levels showed no significant differences in the levels of these hormones before and after ejaculation (Fig. 1A). However, the PGE_2 level of the combined first and second fractions of semen (27.1 ± 5.9 *p*g) was significantly increased in comparison with those of the third fraction collected 0–5 (11.4 ± 2.0 *p*g, $P<0.05$) and 5–10 min (2.9 ± 0.9 *p*g, $P<0.01$) after the start of ejaculation of the third fraction (ANOVA, F=9.67, $P<0.01$). The PGE_2 level of the third fraction collected 0–5 min after the start of ejaculation of the third fraction tended to increase compared with that collected 5–10 min after the start of ejaculation of the third fraction, but the difference was not statistically significant (Fig. 1B).

Table 1. Plasma PGE_2 concentration (mean ± SE, *p*g/m*l*) just before (Pre-PGE_2) and 1 hr after (Post-PGE_2) oral administration of 0.1 mg/kg PGE_2 (n=4)

	Pre-PGE_2	Post-PGE_2
PGE_2 concentration (*p*g/m*l*)	96.8 ± 24.3	2,618.7 ± 740.3*

*P<0.05 for a 2–group comparison.

Table 2. Comparison (mean ± SE) of the combined first and second fraction (1F/2F) and third fraction volumes (3F), total semen volume, total number of sperm, sperm motility, sperm viability and sperm abnormality between untreated control (non-PGE_2) and PGE_2-treated dogs (PGE_2) (n=4)

	Non-PGE_2	PGE_2
1F/2F volume (m*l*)	2.4 ± 0.6	2.5 ± 0.8
3F volume (m*l*)	5.6 ± 1.6	7.8 ± 1.8*
Total semen volume (m*l*)	8.0 ± 2.2	10.3 ± 2.5*
Total number of sperm ($\times 10^8$)	5.8 ± 1.6	8.3 ± 2.0
Sperm motility (%)	79.5 ± 6.6	86.3 ± 2.4
Sperm viability (%)	88.6 ± 3.1	95.0 ± 1.3
Sperm abnormality (%)	8.3 ± 2.0	5.1 ± 1.6

N.S*:* Not significant. *P<0.05 for a 2–group comparison.

In the blood collected before and after oral administration of PGE_2 and semen collected after PGE_2 administration in the 4 dogs, the serum PGE_2 level 1 hr after PGE_2 administration (2,618.7 ± 740.3 *p*g/m*l*) was markedly increased in comparison with that before administration (96.8 ± 24.3 *p*g/m*l*, P<0.05) (Table 1). Moreover, the volume of the third fraction (7.8 ± 1.8 m*l*) and total semen volume (10.3 ± 2.5 m*l*) after PGE_2 administration were significantly increased compared with before administration of PGE_2 (5.6 ± 1.6 m*l*, P<0.05 and 8.0 ± 2.2 m*l*, P<0.05, respectively), but the volume of the combined first and second fractions showed no significant change between before (2.4 ± 0.6 m*l*) and after administration of PGE_2 (2.5 ± 0.8 m*l*). In addition, comparison of total number of sperm, sperm motility, viability and abnormality showed no significant differences between before ($5.8 \pm 1.1 \times 10^8$, 79.5 ± 6.6%, 88.6 ± 3.1% and 8.3 ± 2.0%, respectively) and after PGE_2 administration ($8.1 \pm 2.0 \times 10^8$, 86.3 ± 2.4%, 95.0 ± 1.3% and 5.1 ± 1.6%, respectively) (Table 2).

PGs, such as PGE and $PGF_2\alpha$, are primarily produced in the accessory glands of the reproductive tract and have been shown to affect motility [1, 3, 4, 17], acrosome reaction [15, 16], capacitation, and fertilizing ability of sperm [8, 15]. The present results revealed that PGE_2 production was dramatically increased not in serum but in seminal plasma immediately following ejaculation in male dogs. Previous studies showed no changes in plasma PGE_2 levels in prostatectomized dogs treated with T, but a rise in plasma levels in intact treated dogs [10]. Therefore, PGE_2 is immediately produced and secreted in the prostate gland during the early part of ejaculation.

In addition, PGE_2 administration was found to lead to an increase in the volume of seminal plasma compared with the untreated control. PGE_2 binds to a family of specific E-prostanoid (EP) receptors, including EP1, EP2, EP3 and EP4 receptors. In particular, the action of PGE_2 on EP2 and EP4 receptors was found to stimulate adenylate cyclase and cause relaxation of vascular smooth muscles, leading to the dilatation of blood vessels [12, 13]. Moreover, excitatory activity of EP1 and EP3 receptors produced smooth muscle contraction, which was mediated by Ca^{2+} mobilization or inhibition of adenylate cyclase [13]. In another study, it was shown that EP receptors, such as EP2 and EP3, were expressed in non-tumorigenic gland cells of human prostate tissue [11]. The present results together with previous findings indicate the possibility that production and secretion of a large quantity of seminal plasma in response to PGE_2 administration are associated with the direct effects of prostatic epithelial cells, smooth muscle contraction around the prostate gland and/or vasodilating action. In addition, no significant change in semen quality, including sperm motility, was found in the present study in response to PGE_2 administration. Sperm velocity increases after incubation for 3 hr in the presence of PGE_2 [1], so the reason why semen motility did not improve following PGE_2 administration might include the time of exposure of sperm to PGE_2.

It is concluded that production and secretion of PGE_2 in the prostate gland increase immediately at the start of ejaculation and that PGE_2 plays an essential role in the ejection of a large quantity of seminal plasma in male dogs.

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