NOTE *Theriogenology*

Therapeutic Effect of Aromatase Inhibitor in Two Azoospermic Dogs with High Plasma Estradiol-17β Levels

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ABSTRACT. Two azoospermic dogs with high plasma estradiol-17β (E_2) levels were subcutaneously injected with an aromatase inhibitor (AI), 4-androstene-4-ol-3,17-dione, 2 mg every other day for 4 weeks. Before the AI treatment the plasma E_2 levels of the two dogs (21 and 22 *p*g/m*l*, respectively) were higher than those of 2 normal dogs (8.1 and 12.3 *p*g/m*l*), and they fell to 11–17 *p*g/m*l* between 1 and 4 weeks after the start of AI treatment. The plasma testosterone levels after the start of AI treatment had increased to 2.1–3.1 *n*g/m*l*. A small number of sperm were detected in the semen of the two dogs between 3 and 6 weeks after the start of AI treatment. Th ese results indicate that the testicular function of infertile dogs with high plasma E_2 levels can be temporarily improved by AI therapy.
KEY WORDS: aromatase inhibitor, azoospermia, canine.

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A portion of the testosterone (T) produced by the testis is converted to estradiol-17β (E_2) by aromatase enzyme activity [11]. In the testes of many species [2, 17], including dogs [3], the E_2-secreting cells are the Sertoli cells and/or the Leydig cells [13, 20]. In men [6, 19] abnormally increased testicular E_2 production causes spermatogenic dysfunction, and long-term E_2 administration has been shown to inhibit spermatogenesis in dogs [8, 15]. Aromatase inhibitor (AI) blocks the aromatization of androgen to E_2 by inhibiting aromatase enzyme activity [1, 18], and AI has been reported to be effective in treating spermatogenic dysfunction in men with high plasma E_2 levels [4]. In the present study, two azoospermic dogs with abnormally increased plasma E_2 levels were treated with AI to improve poor semen quality. Plasma E_2 levels after administration of follicle stimulating hormone extracted from porcine pituitary gland (FSH-P) were measured to investigate the main E_2-secretory cells in canine testes.

The two azoospermic dogs used to assess the effects of AI treatment were a Miniature Poodle (Dog 1) and a Beagle (Dog 2), aged 5 and 2 years, respectively. Dog 1 was owned by a breeder, and Dog 2 was cared for in our university. None of a few bitches mated with either of the two male dogs had conceived. Four semen specimens were collected by digital manipulation with a teaser bitch at one-week intervals. Each specimen was examine for total semen volume, total number of sperm, and morphologically abnormal sperm by the methods described previously [9]. As no sperm had been found in the semen of either Dog 1 or Dog 2 (Table 1), both of them were diagnosed as having azoospermia. Although the two azoospermic dogs were treated with 3 intramuscular injections of 1000 IU hCG per head at one-week intervals, their semen quality had failed to improve. Peripheral blood samples were collected from the two azoospermic dogs at the same time as the semen collections. Plasma E_2 and T levels were measured by the radioimmunoassay described previously [8]. The plasma E_2 levels of the two dogs (21 and 22 *p*g/m*l*, respectively) were higher than those of two normal Beagles (Dog A and Dog B, as controls)(8.1 and 12.3 *p*g/m*l*, respectively), aged 2 and 4 years, and their plasma T levels (1.2 and 1.4 *n*g/m*l*) were lower (Table 2).

Semen samples from the two azoospermic dogs were collected at one-week intervals from 1 week before to 8 weeks after the start of injection with AI. Peripheral blood samples were collected from the two azoospermic dogs at the same time as the semen collections. AI, 4-androstene-4-ol-3,17-dione (Sigma Co., Ltd., U.S.A.) was used in this study, and

Table 1. Semen quality[a)] (mean ± S.E.) before aromatase inhibitor administration in 2 dogs with azoospermia (Dog No. 1; a miniature poodle and Dog No. 2; a beagle) and 2 normal beagles (Dog A and Dog B)

Dog No.	Total volume of semen (m*l*)	Total number of sperm ($\times 10^6$)	Motile sperm (%)	Viable sperm (%)	Abnormal sperm (%)
1	2.8 ± 0.2**	0	–	–	–
2	1.9 ± 0.2**	0	–	–	–
A	11.2 ± 1.5	420.4 ± 38.7	95.3 ± 1.4	96.4 ± 1.8	7.8 ± 0.2
B	14.8 ± 2.3	375.3 ± 41.0	91.7 ± 1.4	93.6 ± 2.2	6.3 ± 0.2

a) The semen samples were collected 4 times at 1 week intervals.
** $p<0.01$, in comparison with 2 normal dogs.

Table 2. Mean peripheral plasma estradiol-17β and testosterone levels[a] before aromatase inhibitor treatment to 2 dogs with azoospermia (Dog No. 1 and Dog No. 2), and 2 normal beagles (Dog A and Dog B)

Dog No.	Estradiol-17β (*p*g/m*l*)	Testosterone (*n*g/m*l*)
1	21.0	1.2
2	22.0	1.4
A	12.3	2.3
B	8.1	1.8

a) The blood samples were collected in 2 days at one-week intervals.

the doses were prepared by dissolving 2 mg of AI in 1 m*l* of a cottonseed oil. Each of the two azoospermic dogs was subcutaneously injected the AI, 2 mg every other day for 4 weeks. Two normal dogs, A and B, were subcutaneously injected with 2 mg FSH-P (Denka Pharm.Co., Ltd., Japan) daily for 2 weeks, and peripheral blood samples collected 1 day before, and 1, 2, 3 and 4 weeks after the start of FSH-P administration to measure their plasma E_2 levels.

The high plasma E_2 levels and low plasma T levels before AI treatment decreased and increased, respectively, between 1 and 4 weeks after the start of AI treatment (Fig. 1), and the plasma E_2 (11–17 *p*g/m*l*) and T levels (2.1–3.1 *n*g/m*l*) of the two dogs temporarily reached the normal ranges shown in Table 2. A small number of sperm were first observed in the semen collected from Dog 1 and Dog 2, which had azoospermia, 3 weeks after the start of AI treatment (Fig. 2). The total numbers of sperm in Dog 1 and Dog 2 at 5 weeks after the start of AI treatment were 8.1×10^6 and 22.0×10^6, respectively. High percentages of the sperm had poor motility and a bent or coiled tail (Fig. 2), but no sperm were detected in the semen of either Dog 1 or Dog 2 after 7 weeks of AI treatment. The plasma E_2 levels of the normal dogs A and B after the start of FSH-P administration remained unchanged after hormone administration (Table 3).

The Sertoli cells and Leydig cells of the rat testis are able to produce E_2 [7, 13]. E_2 secretion by Sertoli cells increases after FSH administration in the rat [13], but there is no change in E_2 production after FSH administration in the rabbit testis [17]. Some types of canine Sertoli cell tumors are known to secrete a large volume of E_2 [10, 12]. In the present study, there were no changes in the plasma E_2 levels of the normal male dogs after injection with FSH-P. Therefore, this result suggests that the E_2 secreted by the normal canine testis is mainly produced by the Leydig cells.

It was assumed that the spermatogenic dysfunction of the two dogs in this study was caused by the increased E_2 secretion and decreased T secretion by their testes, the same as in some cases of infertility in men [6, 20]. Testicular E_2 content in the pig [2] and testicular aromatase enzyme activity in the rat [1] increase after hCG administration. The hCG treatment had not been effective in either of the two azoospermic dogs in this study. It has been reported that plasma E_2 levels increased after gonadotropin treatment to infertile men with high plasma E_2 levels [19]. Its failure to exert a therapeutic effect was presumably attributable to the increased E_2 secretion in the dog's testes caused by hCG treatment. A decrease in plasma E_2 levels and increase in plasma T levels have been reported in men [19] and rats [1, 17] after AI treatment. Presumably the AI treatment of the two azoospermic dogs in this study induced the decrease in E_2 secretion and increase in T secretion by the Leydig cells by inhibiting aromatase enzyme activity. The latter half of spermatogenesis in the seminiferous tubules is maintained by T [16]. In the present study, the appearance of sperm in the semen collected from the two dogs 3 weeks after the AI treatment is thought to be induced by transient improvement

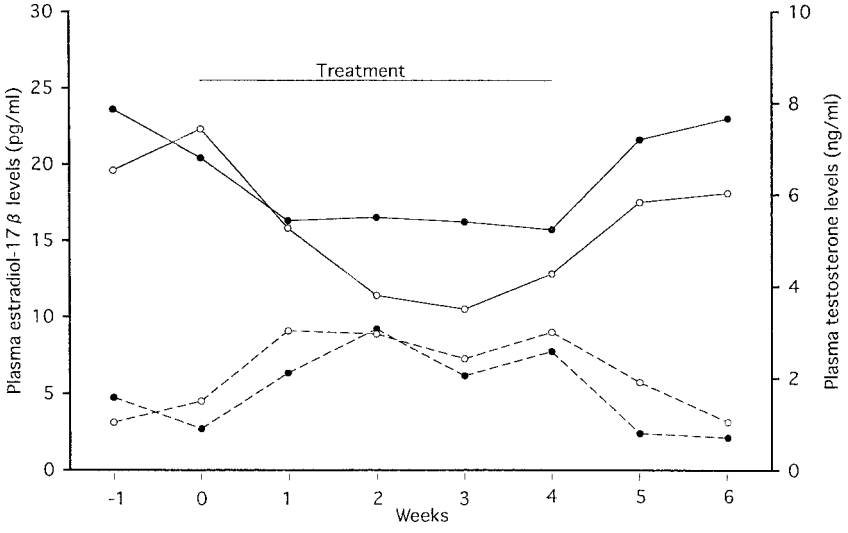

Fig. 1. Changes in peripheral plasma estradiol-17β (—) and testosterone (•••) levels in the two azoospermic dogs (Dog No. 1: ●, Dog No. 2: ○) after the start of aromatase inhibitor treatment.

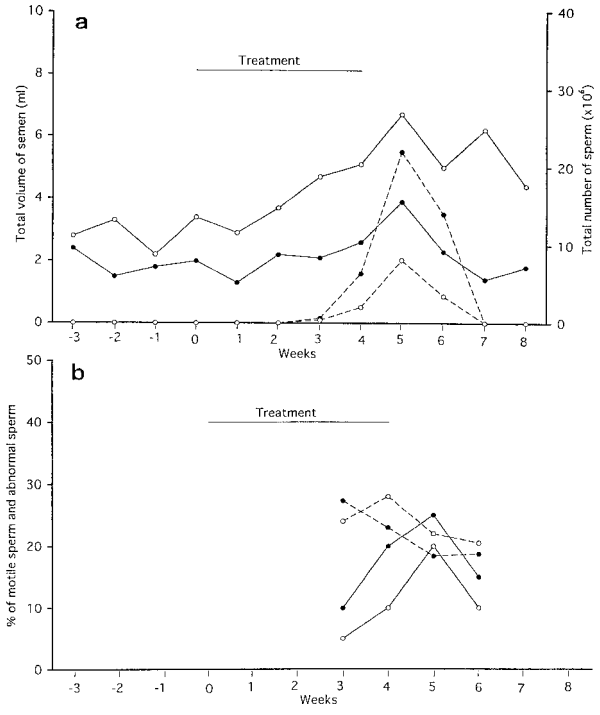

Fig. 2. Changes in semen quality in the two azoospermic dogs (Dog No. 1: ●, Dog No. 2: ○) after the start of aromatase inhibitor treatment. a: Total volume of semen (m*l*) (—) and total number of sperm ($\times 10^6$)(•••). b: Percentages of motile sperm (—) and morphologically abnormal sperm (•••).

of spermatogenesis by the increased T volume in the testes. The duration of spermatogenesis in the dog has been reported to be 54.4 days [5], and the duration of transit of sperm through the epididymis in many mammalian species is 8–14 days [14]. Therefore it is assumed that a small number of sperm had already been presented in a few seminiferous tubules before AI treatment. In the future it will be necessory to investigate the therapeutic effect of longer-term AI treatment and AI plus another hormone treatment in the infertile male dogs with abnormally high plasma E_2 levels.

Table 3. Peripheral plasma estradiol-17β levels (*pg*/m*l*) after the start of FSH-P administration (2 mg/day for 2 weeks) to 2 normal male beagles (Dog A and Dog B)

After administration	Dog A	Dog B
-1 day	10.3	10.8
1 week	8.6	9.2
2 weeks	9.2	8.8
3 weeks	9.0	10.5
4 weeks	8.3	9.7

REFERENCES

1. Akiyama, H. 1997. *Jpn. J. Urol.* **88**: 649–657 (in Japanese with English summary).
2. Allrick, R.D., Christenson, R.K., Ford, J.J. and Zimmerman, D.R. 1983. *Biol. Reprod.* **28**: 902–909.
3. Awoniyi, C., Hasson, T., Chandrashekar, V., Falvo, R.E. and Schanbacher, B.D. 1986. *J. Androl.* **7**: 234–239.
4. Dony, J.M.J., Smals, A.G.H., Rolland, R., Fauser, B.C.J.M. and Thomas, C.M.G. 1986. *Andrologia* **18**: 69–78.
5. Foote, R.H., Swierstra, E.E. and Hunt, W.L. 1972. *Anat. Rec.* **173**: 341–352.
6. Forti, G., Giusti, G., Pazzagli, M., Fiorelli, G., Borrelli, D., Cicchi, P., Guazzelli, R., Conti, C., Scarselli, G., Franchini, M., Boninsegni, R., Mannelli, M. and Serio, M. 1981. *Int. J. Androl.* **4**: 161–171.
7. Genissel, C., Levallet, J. and Carreau, S. 2001. *J. Endocrinol.* **168**: 95–105.
8. Kawakami, E., Amemiya, E., Namikawa, K., Kashiwagi, C., Hori, T. and Tsutsui, T. 2001. *J. Vet. Med. Sci.* **63**: 407–412.
9. Kawakami, E., Tsutsui, T., Yamada, Y. and Yamauchi, M. 1984. *Jpn. J. Vet. Sci.* **46**: 303–308.
10. Lindberg, R., Jonsson, O.J. and Kasstrom, H. 1976. *J. Small Anim. Pract.* **17**: 451–458.
11. Macdonald, P.C., Madden, J.D., Brenner, P.F., Wilson, J.D. and Siiteri, P.K. 1979. *J. Clin. Endocrinol. Metab.* **49**: 905–916.
12. Metzger, Jr., F.L. and Hattel, A.L. 1993. *Canine Pract.* **18**: 32–38.
13. Pomerantz, D.K. 1979. *Biol. Reprod.* **21**: 1247–1255.
14. Rowley, M.J., Teshima, F. and Heller, C.G. 1970. *Fertil. Steril.* **21**: 390–396.
15. Schwartz, E., Tornaben, J.A. and Boxill, G.C. 1989. *Toxicol. Appl. Pharmacol.* **14**: 487–494.
16. Steinberger, E. and Duckett, G.E. 1965. *Endocrinology* **76**: 1184–1189.
17. Thompson, Jr., D.L., Ewing, L.L. and Lasley, B.L. 1965. *Endocrinology* **76**: 1184–1189.
18. Turner, K.J., Morley, M., Atanassova, N., Swanston, I.D. and Sharpe, R.M. 2000. *J. Endocrinol.* **164**: 225–238.
19. Vigersky, R.A. and Glass, A.R. 1981. *J. Clin. Endocrinol. Metab.* **52**: 897–902.
20. Wu, F.C.W., Swanston, I.A. and Bairsd, D.T. 1982. *Clin. Endocrinol.* **16**: 39–47.