



## NOTE

Theriogenology

# Therapeutic effects of oral clomiphene citrate in 2 dogs with low plasma testosterone levels and poor semen quality

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**ABSTRACT.** Two dogs with low plasma testosterone (T) levels and poor semen quality were administered one tablet of 12.5 mg clomiphene citrate orally per day at 2-day intervals for 4 weeks. Plasma T levels, total sperm count, and sperm motility in both dogs temporarily increased between 3 and 6 weeks after the start of treatment. These results indicate that poor semen quality in dogs with low plasma T level can be improved by oral administration of clomiphene citrate.

**KEY WORDS:** clomiphene citrate, oral administration, semen quality, spermatogenic dysfunction, testosterone

We have previously reported that injections of gonadotropin-releasing hormone (GnRH)-analogue [7, 8], human chorionic gonadotropin [5], and pregnant mare's serum gonadotropin plus testosterone (T) [6] are effective for improving semen quality in dogs with low plasma T levels. However, these hormonal injections must be administered subcutaneously or intramuscularly in a small animal hospital.

There have been a few reports describing oral clomiphene citrate treatment for spermatogenic dysfunction in men with low plasma T [1, 11]. Clomiphene citrate is an anti-estrogen that indirectly stimulates secretion of gonadotropic hormones (follicle-stimulating hormone and luteinizing hormone) from the anterior pituitary gland by blocking estrogen receptors in the hypothalamus, which increases the release of GnRH [1, 11]. This increase in plasma gonadotropic hormone levels is thought to induce improved T secretory function of Leydig cells and spermatogenic function in the testes. If oral clomiphene citrate for treatment of spermatogenic dysfunction with low plasma T level is effective in dogs as in men [1, 11], clomiphene citrate can be orally administered by dog owners at home without the need for injection by a veterinarian in a small animal hospital.

In this study, we investigated the efficacy of oral administration of clomiphene citrate as a means of treating poor quality of ejaculated semen in 2 dogs with low plasma T levels.

The 2 dogs treated with oral clomiphene citrate were mongrels aged 4 years (Dog No.1) and 6 years (Dog No.2) (weight: 8 and 10 kg, respectively). Both dogs had been diagnosed with low plasma T levels and poor semen quality based on examinations of plasma and semen samples collected 4 times at 1-week intervals (Table 1). Normal levels of plasma T and normal semen quality in dogs were based on reported mean values in 2 healthy beagles and 3 mongrels (3–6 years old and 7–12 kg body weight): T level, 4.3 ng/ml; total volume of semen, 11.2 ml; total sperm count,  $558.5 \times 10^6$ ; actively motile sperm, 82.3%; and morphologically abnormal sperm, 3.6% [4]. The 2 dogs were cared for in our university, housed in pens with ample runs, and were maintained according to the guidelines of Nippon Veterinary and Life Science University.

The clomiphene citrate used in this study (Clomid, Fuji Pharmaceutical Co., Ltd., Toyama, Japan) was a non-steroidal drug indicated for induction of ovulation in women. The administered dose of clomiphene citrate was decided in reference to a previous report [1]. The 2 dogs were administered one tablet orally containing 12.5 mg clomiphene citrate per day at 2-day intervals for 4 weeks.

Heparinized blood samples were collected from superficial leg veins of both dogs at 2-week intervals from 2 weeks before to 8 weeks after the start of clomiphene citrate treatment. Blood was sampled at 3 different times during the day (9:00, 13:00, and 17:00) because of diurnal fluctuations in plasma T levels in dogs [10]. Plasma T levels were measured with a Testosterone Enzyme-Immunoassay Kit (Cayman Chemical Co., Ann Arbor, MI, U.S.A.) as described previously [4].

Semen specimens were collected once weekly from 2 weeks before to 8 weeks after the first treatment by digital manipulation without a teaser bitch. Each sample was examined for total volume of semen, total sperm count, and percentage of actively motile sperm and morphologically abnormal sperm as previously described [9].

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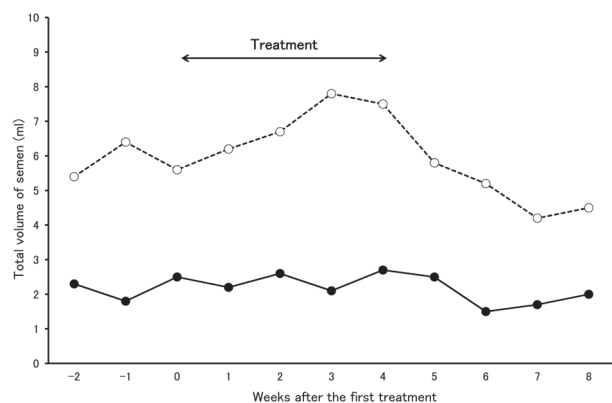


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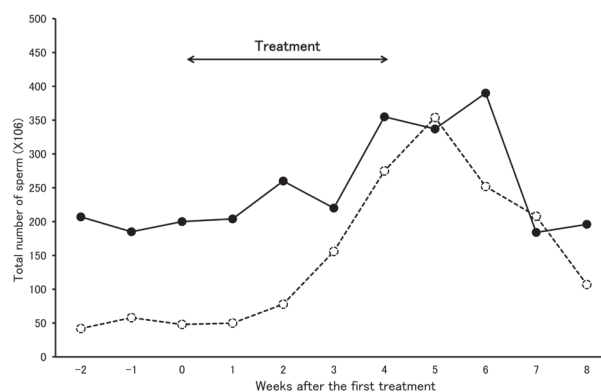
**Table 1.** Plasma testosterone levels and semen quality before clomiphene citrate treatment in 2 dogs with low plasma testosterone and poor semen quality

Dog No.	Testosterone (ng/ml)	Total volume of semen (ml)	Total number of sperm ( $\times 10^6$ )	Motile sperm (%)	Abnormal sperm (%)
1	1.8 $\pm$ 0.2	2.2 $\pm$ 0.3	197.3 $\pm$ 9.2	53.3 $\pm$ 2.4	11.8 $\pm$ 0.8
2	1.5 $\pm$ 0.1	5.8 $\pm$ 0.4	49.3 $\pm$ 6.6	41.7 $\pm$ 2.4	9.5 $\pm$ 1.1

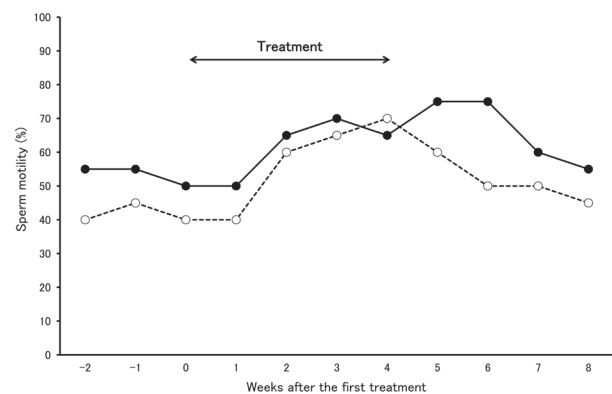
Values are mean  $\pm$  standard deviation. Semen samples were collected 4 times at 1-week intervals.



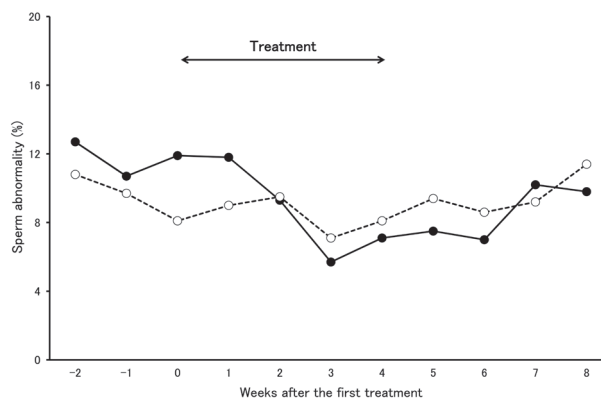
**Fig. 1.** Changes in plasma testosterone levels (ng/ml) of the 2 dogs (Dog No. 1: —●— and Dog No. 2: ○) with poor semen quality between 2 weeks before and 8 weeks after the first treatment.



**Fig. 2.** Changes in total semen volume (ml) of the 2 dogs (Dog No. 1: —●— and Dog No. 2: ○) with poor semen quality between 2 weeks before and 8 weeks after the first treatment.



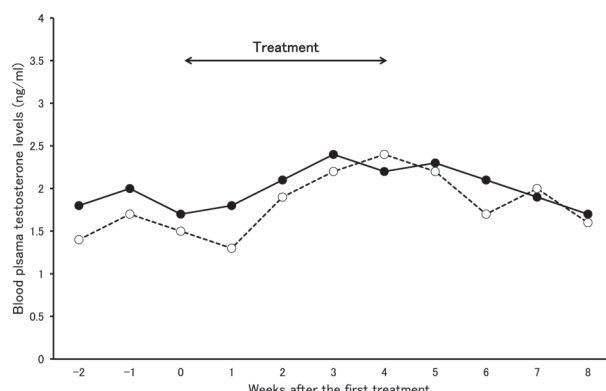
**Fig. 3.** Changes in total sperm count ( $\times 10^6$ ) of the 2 dogs (Dog No. 1: —●— and Dog No. 2: ○) with poor semen quality between 2 weeks before and 8 weeks after the first treatment.



**Fig. 4.** Changes in actively motile sperm (%) of the 2 dogs (Dog No. 1: —●— and Dog No. 2: ○) with poor semen quality between 2 weeks before and 8 weeks after the first treatment.

Plasma T levels in the 2 dogs increased slightly between 2 and 5 weeks after the first treatment (Fig. 1). Although the total semen volume in Dog No.1 did not change after the first treatment, semen volume in Dog No. 2 increased at 3 and 4 weeks after the first treatment (Fig. 2); the total sperm count and percentage of actively motile sperm temporarily increased between 3 and 6 weeks after the first treatment (Figs. 3 and 4). The percentage of morphologically abnormal sperm in both dogs decreased at 3 weeks after the first treatment (Fig. 5).

In this study, it was assumed that the temporary increase in plasma T levels in the 2 dogs after the start of clomiphene citrate was caused by improved T secretory function of Leydig cells in the testes due to stimulation from the increased gonadotropic hormones. Enclomiphene contained in the components of clomiphene increased endogenous LH, FSH and T production in men with secondary hypogonadism, and restored total number of sperm through the hypothalamic-pituitary-testicular axis [3]. Moreover, it has been reported that oral administration of clomiphene citrate induces increased T production and improved spermatogenic function of the



**Fig. 5.** Changes in morphologically abnormal sperm (%) of the 2 dogs (Dog No. 1: ● and Dog No. 2: ○) with poor semen quality between 2 before and 8 weeks after the first treatment.

testes in men [1, 11] and mice [2]. We postulate that the increased gonadotropic hormone secretion from the anterior pituitary gland is also the result of the effect of orally administered clomiphene citrate in dogs with spermatogenic dysfunction.

Based on the results of this study, we conclude that poor semen quality in dogs with low plasma T levels can be improved by oral administration of clomiphene citrate. It has been reported that the GnRH-analogue therapy for dogs with poor semen quality requires multiple subcutaneous or muscular injections of GnRH-analogue by a veterinarian in order to restore the number of sperm [7, 8]. On the other hand, the clomiphene citrate tablet can be administered by the dog owner at home without a veterinarian. Additional cases are needed, however, to evaluate the efficacy of orally administered clomiphene citrate in detail and to determine the optimal dose and duration of clomiphene citrate treatment for the purpose of improving semen quality.

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