NOTE *Theriogenology*

Changes in Plasma Testosterone Level and Semen Quality after Frequent Injections of GnRH Analogue in a Beagle Dog with Azoospermia

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ABSTRACT. A Beagle with a low plasma testosterone (T) level and azoospermia was given 10 subcutaneous injections of 1 μg gonadotropin-releasing hormone analogue (GnRH-A) per head at intervals of 3 days (Experiment 1), and 6 months after the final injection was given, 15 subcutaneous injections of 2 μg GnRH-A were given at intervals of 2 days (Experiment 2). The plasma T level increased and peaked at 8 weeks after the first injection of GnRH-A in both Experiment 1 and Experiment 2. Motile sperm were detected in the semen collected 8 weeks and 7 weeks after the first injection in Experiment 1 and Experiment 2, respectively. The total number of sperm peaked 9 weeks after the first injection in both Experiment 1 (4.5×10^6) and Experiment 2 (72.8×10^6).

KEY WORDS: azoospermia, canine, GnRH analogue.

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The authors have reported using hCG [6] and aromatase inhibitor [5] hormone therapies to treat azoospermia in the dog, but neither therapy has a marked effect on canine azoospermia. In the present study, the authors investigated the efficacy of frequent injections of gonadotropin-releasing hormone analogue (GnRH-A) as a means of treating azoospermia in a dog with a low plasma testosterone (T) level.

The dog treated with GnRH-A was a 4 year-old Beagle cared for at our university. The dog had been fertile until 2 years previously, but when none of the bitches recently mated with the dog conceived, the dog was diagnosed with azoospermia based on a semen evaluation, and the efficacy of GnRH-A therapy was assessed. This study was performed according to the guidelines of the Animal Care and Use Committee of the Nippon Veterinary and Life Science University.

Four weeks before the first injection of GnRH-A in Experiment 1, the right testis was biopsied by collection of tissue with a scalpel under inhalation anesthesia. The tissue was stamped onto a glass slide, and the germ cells on the slide were stained with Giemsa's stain solution.

The GnRH-A used in this study was GnRH ethylamide (D-Ser-tBu-des-Gly-NH2; Buserelin, Hoechst Inc., Germany). The dog was given 10 subcutaneous injections of 1 μg GnRH-A per head at intervals of 3 days (Experiment 1), and 6 months after the final injection in Experiment 1, the dog was given 15 subcutaneous injections of 2 μg GnRH-A per head at intervals of 2 days (Experiment 2) to investigate the efficacious dose of GnRH-A injections for treatment of canine azoospermia. From 2 weeks before to 16 weeks after the first injection of GnRH-A in Experiment 1 and Experiment 2, peripheral plasma and semen samples were collected at 2-week and 1-week intervals. Since the plasma T level of the dog fluctuates diurnally [14], a blood sample was collected 4 times daily (9:00, 12:00, 15:00, 18:00), the 4 plasma T levels for each day were averaged. The plasma T levels were measured with a Testosterone Enzyme-immunoassay Kit (Cayman Chemical Company, MI, U.S.A.). The detection limit of this T assay kit was 10 *p*g/m*l*. Semen quality was examined by methods described previously [9].

Small numbers of primary spermatocytes containing large nuclei were seen on the glass slide stamped with the testicular tissue before treatment, but no spermatids or spermatozoa were observed.

The changes in peripheral plasma T levels in Experiment 1 and Experiment 2 are shown in Fig. 1. The plasma T levels increased and peaked 8 weeks after the first injection of GnRH-A in both Experiment 1 and Experiment 2.

The changes in semen quality in Experiment 1 and Experiment 2 are shown in Figs. 2–5. The total semen volume gradually increased and peaked 9 weeks and 8 weeks after the first injection of GnRH-A in Experiment 1 and Experiment 2, respectively. In Experiment 1, a small number of motile sperm appeared in the semen collected at 8 weeks after the first injection, and the total number (4.5×10^6) of sperm peaked 9 weeks after that. In Experiment 2, however, motile sperm appeared in the semen at 7 weeks after the first injection, and the total number of sperm that appeared 9 weeks after the first injection was higher (72.8×10^6) than the value in Experiment 1. No sperm were detected in the semen at 11 weeks and 15 weeks after the first injection in Experiment 1 and Experiment 2, respectively.

The percentages of actively motile sperm and morphologically abnormal sperm (mainly sperm with a bent tail or coiled tail) were the highest and lowest, respectively, 9 weeks after the first injection in both experiments.

Although some cases of spermatogenic dysfunction in the

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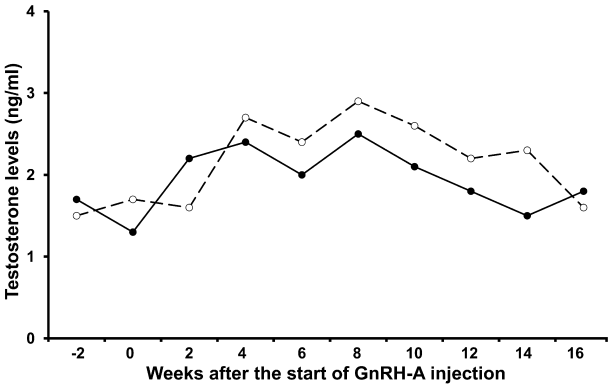

Fig. 1. Peripheral plasma testosterone level (*n*g/m*l*) of the male dog between 2 weeks before and 16 weeks after the first injection of GnRH analogue in Experiments 1 (–●–)and 2 (····○····).

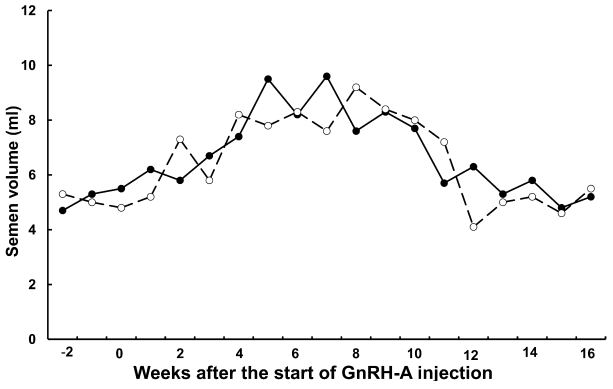

Fig. 2. Total volume (m*l*) of semen of the male dog between 2 weeks before and 16 weeks after the first injection of GnRH analogue in Experiments 1 (–●–)and 2 (····○····).

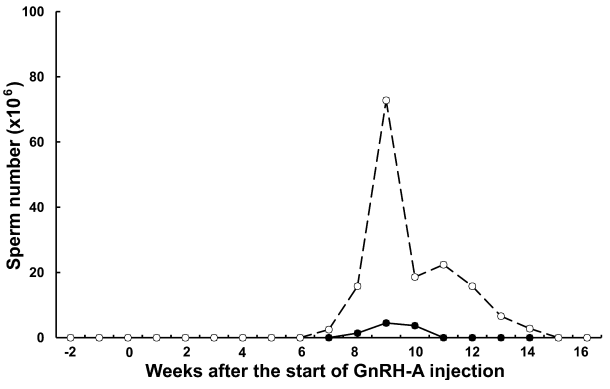

Fig. 3. Total number (× 10^6) of sperm of the male dog between 2 weeks before and 16 weeks after the first injection of GnRH analogue in Experiments 1 (–●–)and 2 (····○····).

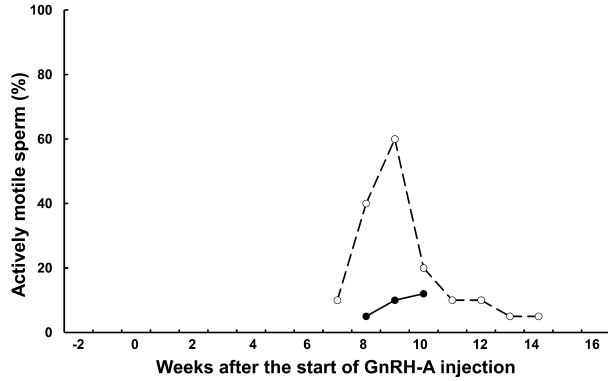

Fig. 4. Percentage of actively motile sperm of the male dog between 2 weeks before and 16 weeks after the first injection of GnRH analogue in Experiments 1 (–●–)and 2 (····○····).

dog have been caused by orchitis [1,4] and endocrine dysfunction [5–8], in many cases of spermatogenic arrest the cause is unknown [2, 3, 10, 11]. The mean plasma T level in normal dogs is 2.05 ± 0.24 (S.E.) *n*g/m*l* [5]. The low plasma T level before GnRH-A treatment in the dog in the present study is thought to have been related to the azoospermia. It was assumed that the poor gonadotropic hormone (GTH) secretory function of the anterior pituitary gland in this dog caused the decrease in T production in the testes. However, the underlying etiology of the occurrence of the GTH secretory arrest in the dog is unknown. The authors have treated dogs with oligo-, astheno- and teratozoospermia using 3 weekly injections of the same GnRH-A (1 μg /kg body weight), and the semen quality of the dogs transiently improved after the course of GnRH-A therapy [8]. There have been no reports of GnRH-A therapy inducing the appearance of motile sperm in semen ejaculated by dogs with azoospermia. In the present study, appearance of motile sperm in the semen of the dog was induced by fre-

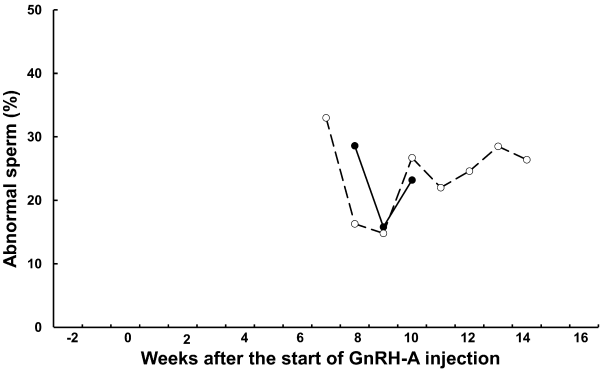

Fig. 5. Percentage of morphologically abnormal sperm of the male dog between 2 weeks before and 16 weeks after the first injection of GnRH analogue in Experiments 1 (–●–)and 2 (····○····).

quent injections of a small dose of GnRH-A, and the injections of 2 μg GnRH-A were more efficacious for improvement of semen quality than 1 μg GnRH-A. The frequent injections are assumed to have induced continuous high levels of secretion of gonadotropic hormones (FSH and LH) and T by the anterior pituitary gland and testes, respectively, in this dog, and especially, the high T level is thought to have stimulated the latter half of spermatogenesis (differentiation from primary spermatocytes) [12] in this dog.

Since it has been reported that fresh semen for intravaginal artificial insemination of bitches should contain 200×10^6 sperm to obtain a conception rate similar to that in naturally breeding bitches [13], it is necessary to investigate the efficacy of sperm after longer-term GnRH-A therapy for the purpose of producing a sperm density greater than 200×10^6.

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