NOTE

Theriogenology

Changes in Plasma Testosterone Levels and Semen Quality after 3 Injections of a GnRH Analogue in 3 Dogs with Spermatogenic Dysfunction

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ABSTRACT. Three male Beagles with low plasma luteinizing hormone and testosterone levels and spermatogenic dysfunction (SD-dogs) were given 3 weekly subcutaneous injections of a gonadotropin-releasing hormone analogue (Buserelin; GnRH-AB). The plasma T level of all of the SD-dogs increased and peaked at 4–6 weeks after the first injection. The total number of sperm and the sperm motility of the three SD-dogs increased 5–7 weeks after the first injection. Therefore, the authors concluded that 3 weekly injections of GnRH-AB transiently improve semen quality in some cases of canine spermatogenic dysfunction.

KEY WORDS: canine, GnRH analogue, spermatogenic dysfunction.

The cause of spermatogenic dysfunction in dogs is often unknown, even after bacterial examinations, cytopathological semen examinations, and endocrinological tests are performed [2, 13, 14]. Gonadotropin-releasing hormone (GnRH) analogues like Fertirelin acetate [9], human chorionic gonadotropin (hCG) [7], and luteinizing hormone (LH) [3] have been used to treat infertility in dogs with poor testicular function in the testes. Administration of GnRH analogues like Fertirelin acetate in normal dogs causes a release of pituitary LH and a subsequent increase in T secretion in the testes [5, 9]. However, GnRH analogues like Buserelin (GnRH-AB) have a more marked effect on pituitary gonadotropin secretion than other GnRH analogues like Fertirelin acetate, and long term administration in dogs has been found to inhibit LH secretion by the anterior pituitary gland and T secretion by the testes [1, 16, 18]. GnRH has been reported to be more effective than hCG in stimulating T secretion by the testes in normal dogs [15]. In this study, changes in plasma T levels and semen quality in dogs with spermatogenic dysfunction (SD-dogs) after 3 weekly injections of GnRH-AB were assessed to evaluate the effects of GnRH-AB therapy.

All three SD-dogs treated with GnRH-AB were 2- or 3-year-old Beagles cared for at our university. The three dogs had been diagnosed with spermatogenic dysfunction based on their semen quality (total number of sperm: less than 200 × 10⁶; actively motile sperm: less than 50%; and abnormal sperm: more than 10%). Three 2- or 3-year-old normal Beagles (total number of sperm: more than 300 × 10⁶; actively motile sperm: more than 70%; and abnormal sperm: less than 10%) were used as controls. Semen samples were collected by digital manipulation at weekly intervals between 3 weeks before and 10 weeks after the first injection of GnRH-AB. The semen specimens were examined by the methods described previously [10].

The GnRH analogue (GnRH-AB) used in this study was GnRH ethylamide (D-Ser-(tBu)-des-Gly-NH₂; Buserelin, Hoechst Inc., Germany). The 3 SD-dogs and the 3 normal dogs were given a subcutaneous injection of 1 µg GnRH-AB/kg to assess the response of the anterior pituitary gland to GnRH-AB. The SD-dogs were subcutaneously injected with 1 µg/kg of GnRH-AB again 1 and 2 weeks after the first injection to improve their testicular function. Peripheral blood specimens were collected from the SD-dogs and the normal dogs at 30 min intervals from 30 min before to 180 min after the first injection and were also collected from the 3 SD-dogs at -3, 0, 2, 4, 6, 8, and 10 weeks after the first injection. Because of the diurnal fluctuations in the plasma T levels of dogs [4, 17], the blood samples were collected 4 times daily (9:00, 12:00, 15:00, and 18:00). Plasma LH was measured by a double-antibody radioimmunoassay (RIA) using the method of Nett et al. [12], as described previously [8]. Plasma T levels were determined by RIA using the method described by Makino et al. [11]. The data for the three normal dogs are summarized as mean values ± standard error (S.E.).

The mean values for the total volume of semen, total number of sperm, percentage of actively motile sperm, and the pH of total semen in the three SD-dogs (Fig. 3) after GnRH-AB injection were markedly lower than the mean values in the normal dogs (Table 1). The mean percentage of morphologically abnormal sperm in each SD-dog was...
higher (Fig. 3), and most of the morphological defects in the sperm consisted of bent or coiled tails.

The mean plasma LH and T levels in each of the SD-dogs before GnRH-AB injection were markedly lower than in the normal dogs (Fig. 1). The plasma LH levels of all the SD-dogs and normal dogs peaked 30 min after the GnRH-AB injection (Fig. 1-a). However, the LH peak levels of all of the SD-dogs were lower than those of the normal dogs. The mean plasma T level of the normal dogs peaked 90 min after the GnRH-AB injection (Fig. 1-b). The peak of plasma T levels for all 3 SD-dogs, on the other hand, occurred 120 min after the GnRH-AB injection, and their T peak levels were lower than in all 3 normal dogs. The plasma T levels in all SD-dogs gradually increased after 3 weekly injections of GnRH-AB and peaked 4 and 6 weeks after the first injection (Fig. 2).

The semen quality of all SD-dogs improved between 5 and 7 weeks after the first injection (Fig. 3). The total number of sperm in the 3 SD-dogs increased to a peak at 6 and 7 weeks after the first injection (Fig. 3-b). At 8 weeks after the first injection of GnRH-AB, however, the semen quality of all three dogs had deteriorated.

A poor LH secretory function of the anterior pituitary glands in the SD-dogs presumably caused the decrease in T production in their testes. A poor endocrine function of the testes in dogs is also thought to cause spermatogenic dys-
function and abnormal secretory function of the epididymis and prostate [6, 9]. The authors have reported that in semen ejaculated from dogs, increases in sperm with low motility and morphological defects in their tails are related to abnormal pH and osmotic pressure in their epididymal and prostatic fluids [6]. It is thought that the plasma LH peaks induced by the 3 injections of GnRH-AB continuously stimulated the Leydig cells of the testes, and subsequently, T secretion by the testes gradually increased.

Improvement in the pH (and presumably the osmotic pressure) of the epididymal and prostatic fluids and an increase in the percentages of actively motile sperm and morphologically normal sperm are assumed to have been induced by the increase in plasma T level.

High-dose or long-term administration of GnRH-AB to dogs is known to inhibit both gonadotropin secretion by the anterior pituitary gland and spermatogenesis in the testis [1, 16, 18]. Based on the results of this study, the authors concluded that weekly injection of 1 µg/kg GnRH-AB for 3 weeks is capable of transiently improving the semen quality of Beagle dogs with spermatogenic dysfunction by reason of the increase in T secretion of the testes. It seems necessary, however, to determine the optimal dose and duration of GnRH-AB administration to improve the semen quality of dogs with spermatogenic dysfunction and for some large-breeds of dog.

REFERENCES

Fig. 3. Changes in total semen volume (a), total number of sperm (b), percentage of actively motile sperm (c), percentage of morphologically abnormal sperm (d), and pH of total semen (e) of the 3 Beagles with spermatogenic dysfunction (Dogs No. 1, 2, and 3) between 3 weeks before and 10 weeks after 3 weekly 1 µg/kg injections of GnRH analogue (GnRH-A).

