Therapeutic Effect of Frequent Injections of GnRH Analogue in a Beagle with Knobbed Acrosome Abnormality of Sperm

Eiichi KAWAKAMI1)*, Tomoko YAGI1), Masanori KOBAYASHI1) and Tatsuya HORI1)

1)Laboratory of Reproduction, Nippon Veterinary and Life Science University, 1–7–1 Kyonan-cho, Musashino, Tokyo 180–8602, Japan

(Received 6 June 2011/Accepted 31 August 2011/Published online in J-STAGE 14 September 2011)

ABSTRACT. A Beagle dog (3 years old) that ejaculated high percentages (mean ± SE: 29.1 ± 1.2%) of sperm with a knobbed acrosome abnormality and a low number of sperm and that also had a low plasma testosterone (T) level was given 10 subcutaneous injections of 1 μg of gonadotropin-releasing hormone analogue (GnRH-A) at 3-day intervals. The plasma T level and number of sperm increased 12–14 weeks after the first injection. Although the percentages of sperm with knobbed acrosome abnormality did not change after the GnRH-A therapy, the number of sperm and percentage of actively motile sperm increased after the therapy, and a bitch gave birth to 5 healthy puppies after intravaginal artificial insemination with fresh semen collected 14 weeks after the first injection.

KEY WORDS: canine, GnRH analogue, knobbed acrosomal defect, sperm.


Knobbed acrosome abnormality of sperm has been reported in bulls [3], stallions [4], boars [2], rams [12] and dogs [10, 11]. The morphological defect of the sperm head is visible as a protrusion from the apical ridge of the acrosomal cap and a cystic swelling on the acrosomal surface [1, 3, 4, 12]. The knobbed acrosome abnormality occurs during spermiogenesis [1, 12], and especially during the acrosomal development of round spermatids [1] in the testes. The abnormality is associated with low fertility or sterility [1–4, 12], and may be inherited in bulls [1] and dogs [11].

We found a dog (3 years old) that ejaculated semen containing a large number of sperm with the knobbed acrosome abnormality (Fig. 1) in our laboratory’s Beagle colony. In the present study, we investigated the effect of frequent injections of a gonadotropin-releasing hormone analogue (GnRH-A) on the percentage of sperm with the knobbed acrosome abnormality in the dog’s semen.

The GnRH-A used in this study was GnRH ethylamide (D-Ser-tBu-des-Gly-NH2; Buserelin, Hoechst Inc., Frankfurt, Germany). The dog was given 10 subcutaneous injections of 1 μg GnRH-A at 3-day intervals according to the method described previously [6]. From 6 weeks before until 18 weeks after the first injection of GnRH-A, peripheral plasma and semen samples were collected 4 times at 2-week intervals and 7 times weekly, respectively. Since the peripheral plasma testosterone (T) level of normal healthy dogs fluctuates diurnally [14], a blood sample was collected 4 times daily (09:00, 12:00, 15:00, 18:00), and the average of the 4 plasma T levels each day was calculated. The plasma T levels were measured with a Testosterone Enzyme-linked Immunoassay Kit (Cayman Chemical Com-

* Correspondence to: KAWAKAMI, E., Laboratory of Reproduction, Nippon Veterinary and Life Science University, 1–7–1 Kyonan-cho, Musashino-shi, Tokyo 180–8602, Tokyo, Japan.

e-mail: kawakami@nvlu.ac.jp

©2012 The Japanese Society of Veterinary Science

Fig. 1. Two sperm of the Beagle with knobbed acrosome abnormality (arrow) (Rose Bengal stain).
Table 1. Peripheral plasma testosterone level (mean ± SE) and semen quality (mean ± SE) in the Beagle dog with knobbed acrosome abnormality of sperm before the start of GnRH-A injections

<table>
<thead>
<tr>
<th></th>
<th>Testosterone (ng/ml)</th>
<th>Total volume of semen (ml)</th>
<th>Total number of sperm (× 10⁶)</th>
<th>Actively motile sperm (%)</th>
<th>Knobbed acrosome (%)</th>
<th>Abnormal tail (%)</th>
<th>Cytoplasmic droplet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before injection</td>
<td>1.4 ± 0.1</td>
<td>3.4 ± 0.1</td>
<td>113.0 ± 3.8</td>
<td>53.6 ± 1.7</td>
<td>29.1 ± 1.2</td>
<td>14.8 ± 0.5</td>
<td>56.0 ± 3.8</td>
</tr>
</tbody>
</table>

Peripheral plasma and semen samples were collected 4 times at 2-week intervals and 7 times weekly from 6 weeks before the first injection of GnRH-A, respectively.

Fig. 2. Change in peripheral plasma testosterone level in the Beagle after the start of GnRH-A injections.

Fig. 3. Changes in total volume of semen (— —) and total number of sperm (-- - -) in the Beagle after the start of GnRH-A injections.

(2.3 ng/ml) and 14 weeks (2.1 ng/ml) after the first injection. The total semen volume, total number of sperm, and percentage of actively motile sperm increased and peaked 13 or 14 weeks after the first injection (14.2 ml, 318 × 10⁶, and 75%, respectively). The percentages of sperm with a tail abnormality (mainly bent or coiled tails) and sperm with a
cytoplasmic droplet decreased and reached their nadir values 13 or 14 weeks after the first injection (8 and 12%, respectively). Although the percentage of sperm with the knobbed acrosome abnormality remained high after the GnRH-A therapy (21% at 14 weeks after the first injection), the bitch gave birth to 5 normal, healthy puppies 61 days after intravaginal artificial insemination.

Normal plasma T levels and normal semen quality in adult Beagles have been reported to be as follows (mean ± SE): plasma T level, 2.6 ± 0.7 ng/ml; total semen volume, 13.4 ± 1.4 ml; total number of sperm, 462.5 ± 37.8 × 10⁶; percentage of actively motile sperm, 89.4 ± 1.6%; and total percentage of abnormal sperm (mainly tail abnormalities), 9.3 ± 0.8% [5]. Although it has been reported that two of four dogs with knobbed acrosome abnormality had normal fertility [11], the plasma T level and semen quality of the dog in this study was obviously inferior to that of normal dogs. In particular, the morphological sperm abnormalities of the dog were characterized by high percentages of sperm with a knobbed acrosomal cap. The grandfather, father, and the 2 male littermates of the dog in this study had semen of normal quality. The etiology of the poor semen quality in the dog is unknown.

It was assumed that the frequent injections of GnRH-A in the present study induced an increase in gonadotropic hormone secretion by the anterior pituitary gland and, in turn, an increase in T secretion by the dog’s testes, as found in a previous report [8]. Transient improvements in spermatogenesis in the testes and in the pH and osmotic pressure of seminal plasma are thought to have been induced by the effect of the increased T production in the testes. We have previously reported that abnormal values of seminal plasma pH and osmotic pressure in dogs with low plasma T levels were associated with poor motility of sperm and morphologically abnormal tails of sperm [7]. The transient decrease in the percentage of sperm with a cytoplasmic droplet was also thought to have been induced by the increase in plasma T level. However, the high percentage of sperm with a knobbed acrosome defect is caused by abnormal formation of the acrosomal cap during the development of spermatids in seminiferous tubules [1]. The production of sperm with the knobbed acrosome abnormality in the dog in the present study may have no relation with the plasma T level. As we could not concluded that the knobbed acrosome abnormality in this dog in the present study is inherited, it seems necessary to closely investigate the relation between some genetic defects and the occurrence of the knobbed acrosome abnormality in dogs.

It has been reported that fresh semen for intravaginal artificial insemination of bitches should contain 200 × 10⁶ sperm to obtain a good conception rate [12]. The number of sperm used for the artificial insemination in this study was 302 × 10⁶. The findings in this study indicate that the semen quality of dogs whose semen contains a high percentage of sperm with a knobbed acrosome abnormality is capable of transiently improving in response to frequent injections of GnRH.

REFERENCES