NOTE *Theriogenology*

Changes in the Plasma Testosterone Level and Testicular Superoxide Dismutase Activity of 5 Azoospermic Beagles after GnRH Analogue Injections

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ABSTRACT. The peripheral blood plasma testosterone (T) levels and superoxide dismutase (SOD) activity were measured in 5 azoospermic (AZ–) beagles. The mean values in the AZ-dogs were significantly lower than in 7 control beagles ($P<0.001$). Subcutaneous injections of 1 μg/kg GnRH analogue three times weekly in the AZ-dogs induced significant increases in mean T level and SOD activity ($P<0.05$) and improvement in spermatogenesis. Thus, spermatogenic function in the dog appears to be maintained by T and normal SOD activity in the testis.

KEY WORDS: azoospermia, canine, SOD.

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Production of reactive oxygen species (ROS) by testicular tissue [13, 18] and sperm [1, 17] has been reported in male animals. Oxidative stress caused by elevated ROS concentrations induces spermatogenic dysfunction [9] and poor semen quality [10], and high ROS concentrations in the seminal plasma of dogs also causes low sperm motility [15]. Superoxide dismutase (SOD) is known to be the most important antioxidant enzyme in seminal plasma [5], and SOD activity has been detected in canine seminal plasma [2]. Leydig [8] and Sertoli cells [11] have been reported to produce SOD in the testis. Low SOD activity in seminal plasma causes infertility in humans [14]. Although the cause of spermatogenic arrest in the dog is not well understood [4], the authors have reported that GnRH analogue (–A) injection transiently improved the semen quality of some dogs with poor testosterone (T) secretory function in the testes and spermatogenic dysfunction [7]. In the present study the plasma T levels and testicular SOD activities of azoospermic (AZ–) dogs were measured after GnRH-A injections in order to investigate the cause of spermatogenic dysfunction and the interaction between T secretory function and SOD activity in the testes.

Five AZ-beagles (4–7 years old) cared for at our university were used in this study. The 5 dogs had previously been diagnosed with azoospermia based on examinations of ejaculated semen collected by digital manipulation at weekly intervals for 4 weeks. Seven beagles (3–6 years old) with normal semen quality (the total number of sperm was more than 300×10^6, more than 80% of sperm were actively motile, and less than 10% of sperm were abnormal) were used as controls.

The GnRH-A used in this study was GnRH ethylamide (D-Ser-(tBu)-des-gly-NH_2; Buserelin, Hoechst Inc., Germany). The AZ-dogs were given 3 weekly subcutaneous injections of 1 μg GnRH-A per kilogram body weight according to the method described previously [7]. Blood samples were collected from a peripheral vein 5 weeks before and 10 weeks after the first injection of GnRH-A. Since the plasma T level of male dogs fluctuates diurnally [6, 16], blood samples were collected 3 times a day (09:00, 13:00, 17:00). Plasma T levels were determined by radioimmunoassay using the method described by DePalatis *et al.* [3], and the mean T level was calculated for the 3 plasma samples collected each day.

Tissue was collected twice from the right testis of each AZ– and control dog by biopsy under inhalation anesthesia 5 weeks before and 10 weeks after the first injection of GnRH-A. The tissue was stamped onto a glass slide, and the germ cells on the slide were stained with rose bengal solution (3 g rose bengal, 1 m*l* formalin, and 99 m*l* distilled water) for 15 min to identify sperm. The testicular tissue was homogenized, and the suspensions obtained were collected to measure SOD activity with an SOD Assay Kit (Trevigen Inc., MD, U.S.A.). A spectrophotometer was used to measure SOD activity at an absorbance of 550 nm.

The data from the AZ– and control dogs are summarized as mean values ± standard error (SE). Differences between means were analyzed for statistical significance by Student's *t*-test.

The mean values of the peripheral plasma T level and testicular SOD activities of the AZ-dogs were significantly lower than in the control dogs ($P<0.001$; Table 1). The plasma T levels and testicular SOD activities of all the AZ–dogs increased after the GnRH-A injections, and the mean T and SOD values 10 weeks after the first injection of GnRH-A were significantly higher than before the start of the GnRH-A injections ($P<0.05$; Table 1).

No sperm were observed on the glass slides stamped with testicular tissue collected from any of the AZ-dogs before the GnRH-A injections. However, small numbers of morphologically normal sperm were observed on the slides of one (Dog No. 2) of the 5 AZ-dogs 10 weeks after the first injection of GnRH-A. The plasma T level and testicular SOD activity values of Dog No. 2 were the highest among

Table 1. Mean (± SE) plasma T levels and testicular SOD activities of 7 control beagles and 5 azoospermic beagles, before and 10 weeks after the first injection of GnRH-A

	Control dogs	Azoospermic dogs	
		Before	After
T (*n*g/m*l*)	2.34 ± 0.20	0.83 ± 0.14[a)]**	1.31 ± 0.14[b)]*
SOD (unit/g protein)	166.4 ± 20.5	73.3 ± 7.2[a)]**	109.0 ± 13.8[b)]*

a)**: P<0.001, in comparison with the value in the control dogs.
b)*: P<0.05, in comparison with the value before GnRH-A injection.

the 5 AZ-dogs, both before and after the GnRH-A injections (1.08 *n*g/m*l* and 86 units/g protein before and 1.86 *n*g/m*l* and 169 units/g protein after).

Testicular SOD is thought to be an important antioxidant enzyme for maintaining normal spermatogenic and steroid hormone secretory function in the testis [12]. Low SOD activity in the testis induces poor spermatogenic function [18] because of an increase in ROS concentration [9]. The T secretion by the testes and testicular SOD activity in all of the AZ-dogs in the present study increased after the GnRH-A injections. The GnRH-A injections are thought to have stimulated the LH and FSH secretory function of the anterior pituitaries of the AZ-dogs. High plasma levels of LH and FSH stimulate Leydig and Sertoli cells, respectively, in the testis. SOD has been reported that to be produced by both Leydig cells [8] and Sertoli cells [11]. The increase in SOD production in the testes of the AZ-dogs may have been directly caused by the high plasma LH and FSH levels or indirectly caused by the increase in T secretion by Leydig cells.

The highest values of plasma T and testicular SOD activity in the AZ-dogs were found in the dog whose testicular tissue, after being stamped onto a slide, revealed the presence of sperm after the GnRH-A injections (Dog No. 2). Thus, spermatogenic function in the dog appears to be maintained not only by normal T secretion but also by normal SOD activity in the testis.

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