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NOTE

Theriogenology

Effect of supplementation with the reduced form of coenzyme Q10 on semen quality and antioxidant status in dogs with poor semen quality: Three case studies

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ABSTRACT. Oxidative stress owing to an imbalance between reactive oxygen species and antioxidants, such as coenzyme Q10 (CoQ10), is a major contributor to male infertility. We investigated the effects of the reduced form of CoQ10 (ubiquinol) supplementation on semen quality in dogs with poor semen quality. Three dogs received 100 mg of ubiquinol orally once daily for 12 weeks. Semen quality, serum testosterone, and seminal plasma superoxide dismutase (SOD) activity were examined at 2-week intervals from 2 weeks before ubiquinol supplementation to 4 weeks after the treatment. Ubiquinol improved sperm motility, reduced morphologically abnormal sperm, and increased seminal plasma SOD activity; however, it had no effect on testosterone level, semen volume, and sperm number. Ubiquinol supplementation could be used as a non-endocrine therapy for infertile dogs.

KEY WORDS: antioxidant, canine, coenzyme Q10, semen quality, superoxide dismutase

Infertility in male dogs is defined as the inability to impregnate a fertile female dog despite multiple mating near the ovulation period [13]. Poor semen quality is one of the factors responsible for male dog infertility and is caused by congenital anomalies such as cryptorchidism, testicular hypoplasia, hormonal disturbance, testicular or prostatic disorders, infections, and increased reactive oxygen species (ROS) levels in seminal plasma [13, 15, 29, 44]. However, identification of the actual cause is difficult [15].

Endogenously generated ROS, such as hydrogen peroxide, superoxide anions, and hydroxyl radicals, from the testicular tissue, sperm, and seminal fluid leukocytes play an important role in male reproductive functions [34, 37, 38, 41]. ROS cause lipid peroxidation of the plasma membrane, DNA damage in the nucleus and mitochondria, apoptosis in sperm, and deterioration of the physiological functions of spermatozoa [1, 3, 24, 36, 38]. Further, the activity of seminal plasma antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, is known to be reduced in humans and dogs with oligozoospermia, asthenozoospermia, and teratozoospermia [4, 23, 27, 42, 44].

Coenzyme Q10 (CoQ10) is ubiquitously expressed in all cells and is located mainly in the inner mitochondrial membrane. CoQ10 is an essential cofactor in the mitochondrial respiratory chain as an electron carrier from complexes I and II to complex III and is essential for energy metabolism and oxidative activity [32, 46]. CoQ10 also regulates the expression of genes involved in cellular signaling, metabolism, and nutrient transport [17]. CoQ10 exists in the oxidized (ubiquinone) and reduced (ubiquinol) forms, with ubiquinol being a potent lipophilic antioxidant in mitochondrial and lipid membranes [35, 39]. CoQ10 is actively biosynthesized in the testes and is present at high concentrations in the serum, suggesting that it plays an important role in protecting sperm from ROS-induced oxidation [19, 25, 26]. CoQ10 levels in sperm cells and seminal plasma are reported to be lower for infertile men with idiopathic or varicocele-associated asthenozoospermia than for healthy donors with normozoospermia [10]. Furthermore, oral administration of ubiquinol to males with idiopathic oligoasthenoteratozoospermia has been shown to improve semen quality and oxidative status in seminal plasma [5]. However, the influence of CoQ10 supplementation on canine semen is unclear. The purpose of this study was to understand the effect of CoQ10 treatment on semen quality, serum testosterone levels, and seminal plasma SOD activity in dogs with poor semen quality.

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Table 1. Mean (± standard error (SE)) semen quality parameters and serum testosterone levels in samples collected from three dogs before ubiquinol supplementation

Dog no.	Total semen volume (ml)	Total number of sperm ($\times 10^8$)	Progressively motile sperm (%)	Morphologically abnormal sperm (%)	Serum testosterone (ng/ml)
1	6.03 ± 0.79	0.80 ± 0.27	58.33 ± 4.41	14.40 ± 2.00	2.46 ± 0.16
2	14.17 ± 0.19	1.58 ± 0.05	71.67 ± 1.67	11.50 ± 1.68	2.00 ± 0.44
3	1.70 ± 0.20	1.33 ± 0.24	58.33 ± 1.67	17.23 ± 1.75	2.16 ± 0.71

Three mixed-breed dogs (Dogs 1, 2, and 3), all within 10–11 years of age and a body weight of 8–12 kg, were used in this study. The semen quality of these dogs, based on examinations of semen collected three times prior to ubiquinol administration, was found to be inferior to that previously reported in healthy beagle dogs [23]; therefore, these dogs were diagnosed with poor semen quality. The serum testosterone concentrations in these dogs were within the reference range (2–4 ng/ml [20]) (Table 1). All animal experiments were approved by the Experimental Animal Ethics Committee of Nippon Veterinary and Life Science University (approval no. 2020S-33).

The dogs were each administered 100 mg of ubiquinol (Kaneka Your Healthcare, Tokyo, Japan) orally once daily for 12 weeks. The dose of ubiquinol supplementation was determined based on a study on the treatment of human patients with idiopathic oligoasthenoteratospermia [5]. Semen was collected once every 2 weeks from 2 weeks before ubiquinol supplementation to 4 weeks after the treatment via digital manipulation without a teaser female. Each sample was examined for total number of sperm and volume of semen and percentage of actively motile sperm and morphologically abnormal sperm. The total number of sperm was calculated using a hemocytometer; sperm motility was evaluated using a sperm motility examination plate on a warm platform using a previously described method [21]. Morphological abnormalities of sperm were examined by 1% eosin staining and classified according to the sperm region as head, midpiece, or tail defects. The sperm-rich ejaculate fraction of semen was collected from these dogs, centrifuged at 1,500 × *g* for 5 min, and the supernatant was collected. SOD activity in the supernatant was measured using a SOD Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) and a microplate reader (PowerScan HT; DS Pharma Biomedical, Osaka, Japan) at an absorbance of 450 nm according to the manufacturer's protocol. Protein concentration in the supernatant was measured to normalize SOD activity using a BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and the microplate reader at an absorbance of 562 nm; SOD activity was expressed as U/g protein [23]. Blood samples were collected from the cephalic vein four times a day (09:00, 12:00, 15:00, and 18:00) every 2 weeks, considering the diurnal fluctuations in blood testosterone concentrations [45]. Serum testosterone concentrations were measured using a testosterone enzyme-linked immunosorbent assay kit (Cayman Chemical) according to the manufacturer's protocol.

All semen parameters and SOD activity are expressed as the mean ± standard error. A paired samples *t*-test was used to compare the mean values before and after treatment, and *P*-values less than 0.05 were considered statistically significant.

Compared with those in samples collected from beagle dogs with normozoospermia [23], the total number of sperm and the percentage of progressively motile sperm in semen ejaculated by the dogs included in the study were found to be low before the initiation of ubiquinol supplementation, whereas the percentage of morphologically abnormal sperm with a coiled or bent tail was high (Table 1).

The percentage of progressively motile sperm increased gradually after the start of ubiquinol supplementation and increased between 4 and 12 weeks of supplementation ($P<0.05$, and 0.01, respectively). However, this improvement in sperm motility was gradually decreased to pre-supplementation levels by 4 weeks after stopping supplementation (Fig. 1A). There was no significant change in the total volume of semen (Fig. 1B) or the total number of sperm (Fig. 1C) before and after ubiquinol supplementation. Similar to the timing of improvement in sperm motility, there was a significant decrease ($P<0.05$) in the percentage of total abnormal sperm (Fig. 2A) and, particularly, of sperm with tail defects (Fig. 2B). However, the percentage of sperm with midpiece (Fig. 2C) and head defects (Fig. 2D) after ubiquinol supplementation was not significantly different from that before supplementation. Moreover, peripheral blood serum testosterone levels did not change during supplementation (Fig. 3A). SOD activity in the seminal plasma of the sperm-rich fraction was gradually increased after ubiquinol supplementation, with a significant increase at 10 and 12 weeks after the initiation of supplementation ($P<0.01$ and 0.05, respectively) (Fig. 3B). After supplementation was stopped, SOD activity was decreased to the pre-supplementation level.

A large number of mitochondria are present in sperm, and a high level of energy is required during flagellar movement [14]. ROS produced endogenously during sperm metabolism and motility cause loss of sperm membrane integrity by oxidation, resulting in decreased sperm motility, dysfunction, and morphological abnormalities [1, 16, 43]. Antioxidant enzymes such as SOD, glutathione peroxidase, and catalase, are present in seminal plasma; these enzymes act as scavengers that protect cells from oxidative damage by ROS [8, 18, 33]. Ubiquinol, the reduced form of CoQ10, functions as an antioxidant, protecting the membranes of cells including sperm against oxidation, inhibiting lipid peroxidation, and indirectly stabilizing calcium channels [12, 28, 40]. In this study, we evaluated the effect of long-term oral supplementation with ubiquinol on canine semen quality.

Ubiquinol supplementation in dogs with poor semen quality markedly improved the percentage of actively motile sperm and reduced the percentage of abnormal sperm associated with elevated SOD levels in seminal plasma. Previous reports have shown that infertile males have low CoQ10 concentrations and antioxidant activity in semen and sperm cells, resulting in poor semen quality; however, CoQ10 administration improves the antioxidant or oxidant status, sperm motility, and morphology [5, 7, 9, 35].

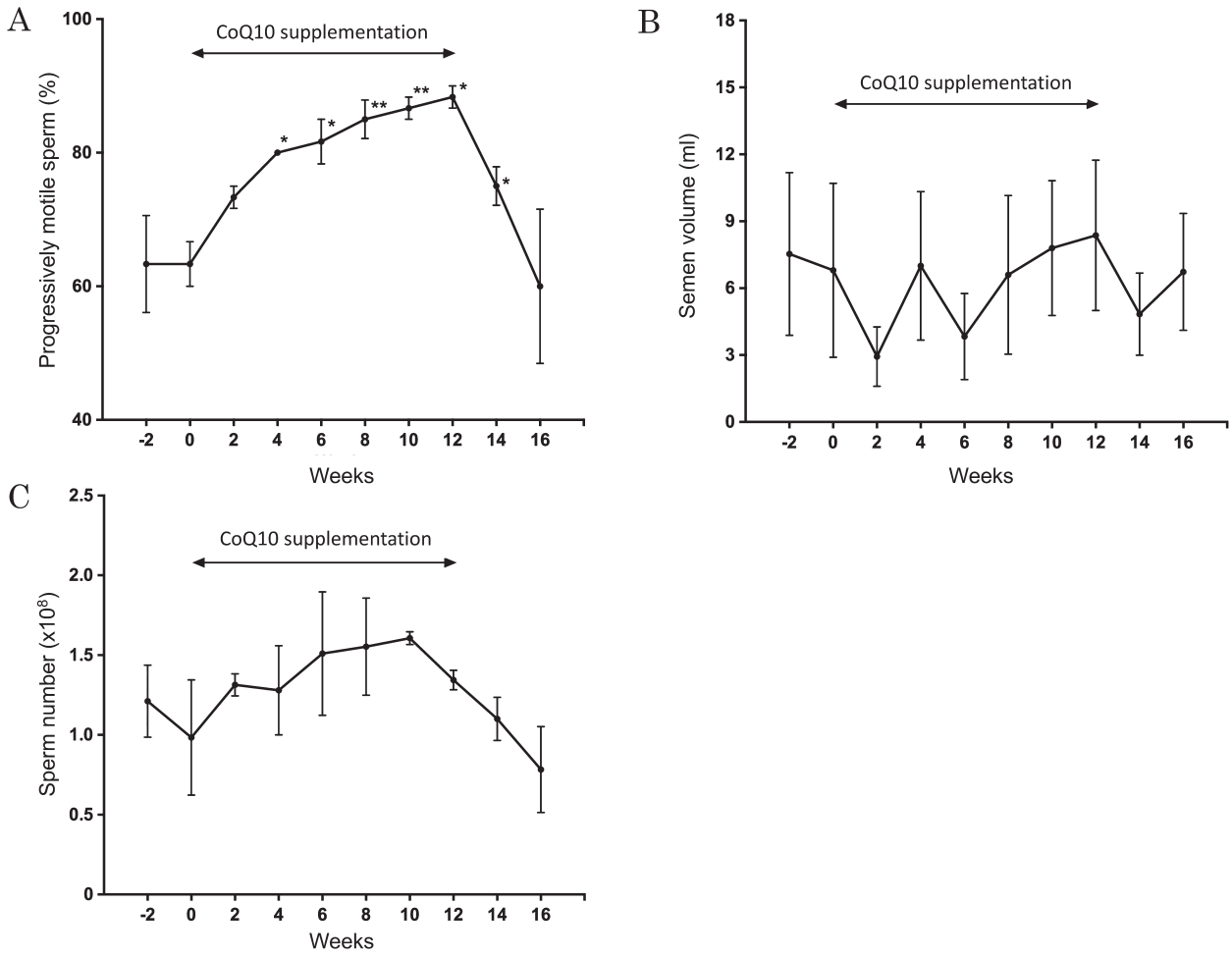

Fig. 1. Changes in the mean (± standard error (SE)) percentage of progressively motile sperm (A), mean (± SE) total volume of semen (B) and total number of sperm (C) collected from three dogs with poor semen quality at 2-week intervals from 2 weeks before ubiquinol supplementation to 4 weeks after the treatment. **P*<0.05, ***P*<0.01, compared to pre-ubiquinol supplementation (0 week).

Moreover, sperm concentration, motility, and morphology after CoQ10 therapy correlated significantly with antioxidants such as SOD in seminal plasma [6]. Asthenozoospermic dogs have a low antioxidant capacity, and administration of the antioxidant vitamin E improves sperm motility in these animals [22, 23]. Conversely, few reports suggest that sperm motility and morphology are not clearly associated with seminal oxidative defense in humans [47, 48]. Our findings suggest that continuous ubiquinol supplementation enhances antioxidant capacity in seminal plasma, thereby improving semen quality, at least in dogs.

Ubiquinol supplementation did not increase the total number of sperm or serum testosterone concentrations in these dogs. However, semen quality, including sperm density, is reported to be increased in human males with idiopathic infertility who receive ubiquinol [35]. Further, CoQ10 has a direct protective effect on testicular tissue in rats [30]. However, CoQ10 supplementation does not induce significant increase in sperm concentration despite alleviation of oxidative stress [31]. Additionally, there is no consensus on the effects of CoQ10 on testosterone levels [11]. The effects of ubiquinol administration on the total number of sperm and on testosterone secretion in dogs may thus need to be reexamined with additional cases considering individual differences. The combination of ubiquinol with other antioxidants, such as vitamin E, may also be effective in improving these parameters [2, 22].

In conclusion, supplementation with ubiquinol in aging dogs with poor semen quality resulted in improved sperm motility, a decreased number of morphologically abnormal sperm, and increased SOD activity in seminal plasma, indicating that it may represent a non-endocrine therapeutic option in male dogs with asthenozoospermia or teratozoospermia. As improvement of semen quality and antioxidant status by ubiquinol treatment was temporary, continuous intake of ubiquinol is necessary. Further research is thus needed to determine the optimal therapeutic objective and doses of ubiquinol. Overall, our results indicate that improving semen parameters of dogs by treatment with ubiquinol may increase their conception rates.

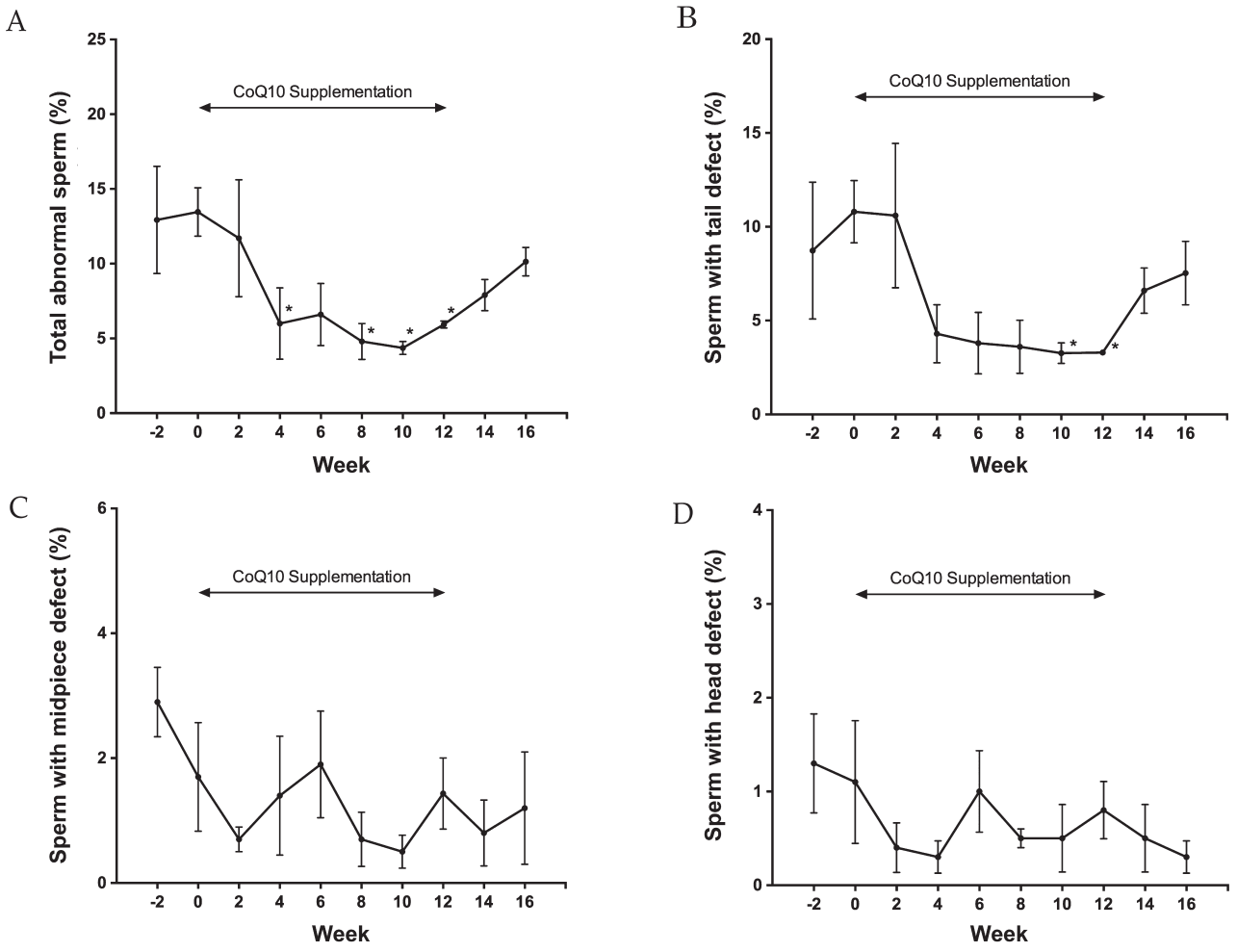

Fig. 2. Changes in the mean (± standard error (SE)) percentage of total abnormal sperm (A), sperm with tail (B), midpiece (C) and head (D) defects collected from three dogs with poor semen quality at 2-week intervals from 2 weeks before ubiquinol supplementation to 4 weeks after the treatment. **P*<0.05, compared to pre-ubiquinol supplementation (0 week).

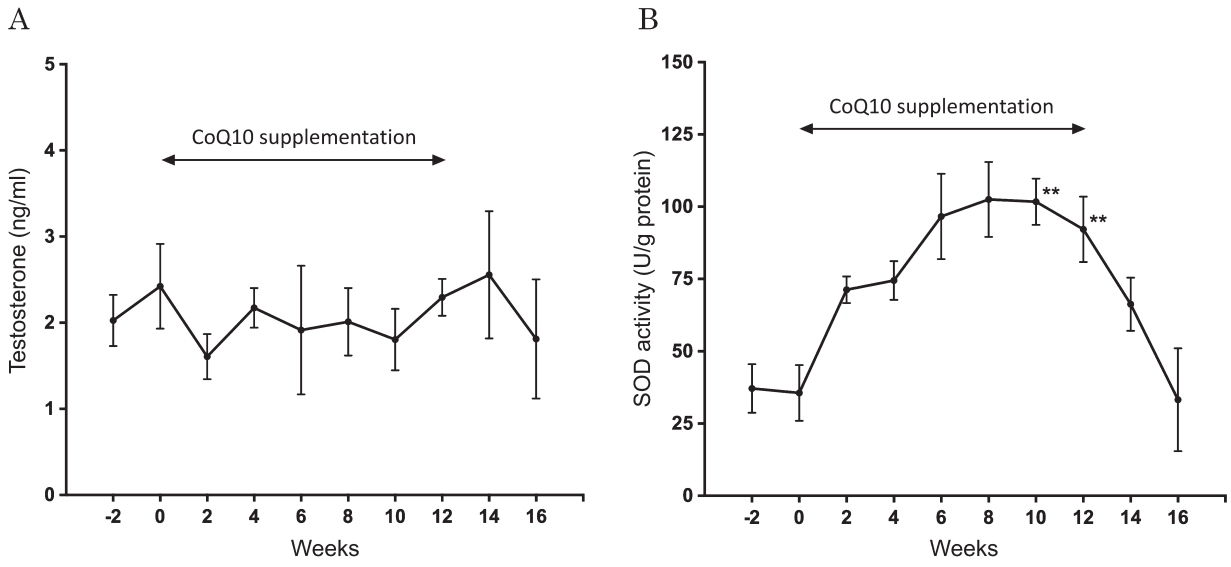

Fig. 3. Changes in mean (± standard error (SE)) serum testosterone levels (ng/ml) (A) and SOD activity in seminal plasma (U/g protein) (B) collected from three dogs with poor semen quality at 2-week intervals from 2 weeks before ubiquinol supplementation to 4 weeks after the treatment. ***P*<0.01, compared to pre-ubiquinol supplementation (0 week).

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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