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Improvement of sperm motility within one month under selenium and vitamin E supplementation in four infertile dogs with low selenium status

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Abstract

Introduction: Significant improvement of sperm motility within one month effected by oral supplementation of selenium and vitamin E was described in four infertile male dogs which failed to conceive in their last three matings with different bitches. **Material and Methods:** The dogs (a Golden Retriever, an English Cocker Spaniel, and two Tibetan Mastiffs) were supplemented daily with selenium (Se) (0.6 mg/kg organic Se yeast) and vitamin E (vit. E) (5 mg/kg) *per os* for 60 days. Semen was collected on days 0, 30, 60, and 90. The sperm concentration and motility parameters were evaluated by the CASA system, sperm morphology was explored by Diff-Quick staining, and live and dead spermatozoa were differentiated by eosin/nigrosin staining. The concentrations of Se and vit. E were measured in peripheral blood serum on semen collection days. **Results:** Before administration, the concentrations of Se in blood plasma were low (86.0–165.0 µg/L). After 30 days of treatment there was an observable improvement in total and progressive sperm motility and kinematic parameters (VAP, VSK, VCL, ALH, BCF, and RAPID). The percentages of live and normal morphology sperm cells were also higher. There was also an observable increase in Se and vitamin E concentrations in blood serum. Bitches were successfully mated and delivered four to six puppies. **Conclusion:** Supplementation with Se and vit. E improved rapid sperm motility and restored fertility in infertile dogs with low Se status.

Keywords: dog, selenium, vitamin E, semen, fertility.

Introduction

Knowledge about infertility in the canine male is still insufficient. Poor semen quality can appear at every age, often with unknown aetiology (11, 12, 20). Sperm production relies on external and internal factors which influence testicular function (4, 12). In gonads, induction of apoptosis and oxidative stress is supposed to be a normal regulatory process of germ cell formation, but the antioxidant defence of spermatozoa is weak in these cells and highly vulnerable to oxidative stress (37). The relationship between oxidative stress, antioxidants, and male fertility has been shown in several studies in humans and other species (1, 2, 40). When dogs are otherwise healthy attention should be paid to provision of an appropriate diet. The dietary quantity of components which affect spermatogenesis can be the key to improving semen quality, especially in circumstances of their deficiency. The well-known pertinent dietary components are the antioxidants: selenium (Se) and vitamin E (vit. E) as well as polyunsaturated fatty acids (5, 6, 7, 40). Se operates through selenoproteins: the main selenoprotein P and glutathione peroxidase (GPx). The first supplies Se to the testes (6) and the second is the major selenoprotein in the testes (5, 22). Vit. E and fatty acids protect spermatozoa against lipid peroxidation, which has critical consequences for sperm cells as an oxidation reaction (40). All these antioxidants act synergistically when administered together.

Data concerning oral supplementation with Se and vit. E and effect on canine semen quality are very limited (10). In this study we have shown a rapid increase in canine sperm motility after just 30 days of supplementation with Se and vit. E followed by restoration of fertility in four infertile males.

Material and Methods

The following males were used in the study: a Golden Retriever (I; 36 kg b.w.), an English Cocker Spaniel (II; 23 kg b.w.), and two Tibetan Mastiffs (III; 62 kg b.w.; and IV; 64 kg. b.w.). They were referred to the Clinic in the Department of Animal Reproduction because of conception failure in their last three matings with different bitches. Before the failed matings these males had at least one litter. The ages of the dogs ranged from three to six years. The patients were in good general condition with normal sexual libido (typical sexual behaviour during natural mating and no problems with semen collection if artificial insemination (AI) was performed), without any disorders or abnormalities of the genital tract, no internal diseases or diseases which raised body temperature were stated in the patients' histories, and no drugs or hormonal treatment had been used. The dogs were fed various commercial dry feeds. The prostate gland and testes were examined by ultrasonography (8.0 MHz probe MyLab30Gold; Esoate, USA) and did not show any pathological condition.

The dogs were supplemented daily with Se (6 μg/kg of organic selenium from yeast) and vit. E (5 mg/kg) per os for 60 days (Semevet; VetExpert, Poland). The preparation also contained 50 mg of evening primrose extract.

Semen was collected by manual manipulation as described by Linde-Forsberg (24) in the presence of a teaser bitch in heat on days 0, 30, 60, and 90. The ejaculates were collected into a prewarmed (36–38°C) glass tube.

The sperm concentration and motility indicators were assessed by an IVOS Sperm Analyser, version 12.3 (Hamilton, USA). Ejaculates were diluted to 50×10^6 spermatozoa/mL with TRIS extender directly before analysis. The following parameters were measured: concentration (CONC), the percentage of motile spermatozoa (MOT), the percentage of spermatozoa with a progressive motility (PMOT), velocity average pathway (VAP), velocity straight line (VSL), velocity curvilinear (VCL), amplitude lateral head (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN), and rapid, medium, slow, and static motility subcategories.

The percentages of live and dead spermatozoa were estimated on dried smears stained with eosin/nigrosin. For the assessment of sperm morphology, monochromatic Diff-Quick stain was used. Two hundred spermatozoa were evaluated per slide, representing 100% of the sample.

Reference values for semen quality parameters were taken from Günzel-Apel *et al*. (13), Rijsalaere *et al*. (32), and Niżański *et al.* (30).

Additionally on semen examination days blood was collected from the cephalic vein and sent directly to the laboratory (in a cooled expanded polystyrene box) to determine serum concentration of Se by inductively coupled plasma mass spectrometry (Aurora M90 ICP-MS, Bruker Corp, USA) and vit. E by high-performance liquid chromatography (Dionex Ultimate 3000 RSLC, Thermo Fisher Scientific, USA).

Statistical analysis was not carried out as the dogs were designated clinical cases.

Results

On day 0 the concentrations of Se in blood plasma were low and ranged from 86.0 to 165.0 μg/L, and those of vitamin E were below the reference values in three males (I, III, and IV). There were significant rises in Se and vit. E levels in the blood plasma of all four males during supplementation (Table 1).

On day 0 the semen motility parameters were low. The results that were obtained on day 30 were surprising because there was a prominent increase in MOT and PMOT, improvement in most motility indicators (VAP, VSL, VCL, ALH, and BCF) and a rise in the subpopulation of spermatozoa with rapid movement (Table 2). The percentages of live and morphologically normal sperm cells were also higher (Table 3). The concentration of spermatozoa was stronger after 60 days of supplementation (Table 2).

Each male mated naturally with or AI with each male's semen was performed in one to four bitches in a period of 70 days from the beginning of supplementation (one month after supplementation). All bitches got pregnant and had four to six puppies in their litters.

Table 1. Selenium and vitamin E concentrations in peripheral blood serum in four males (I, II, III and IV) under supplementation with the same on days 0, 30, 60 and 90

Parameter	Dog															
	I				II				III				IV			
	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90
Selenium (μg/L)	86	279	317	352	165	283	308	298	98	289	316	345	100	300	364	401
Vitamin E (mg/L)	11.5	29.7	42.1	40.1	41.1	42.3	48.9	47.9	15.6	33.3	38.9	42.6	11.2	31.2	31.5	40.2

Table 2. Volume, concentration, and motility parameters of semen in four male dogs (I, II, III and IV) under supplementation with selenium and vitamin E on days 0, 30, 60 and 90

Parameter	Unit	Dog															
		I				II				III				IV			
		Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90
Volume (sperm-rich fraction)	mL	3.0	3.5	3.0	3.9	1.2	1.5	1.5	1.3	3.0	3.5	3.7	3.7	3.2	3.5	3.6	3.5
Concentration	$x10^6$/mL	112.4	132.4	321.3	318.3	138.6	141.2	389.3	392.1	111.9	145.7	299.0	314.8	88.4	128.0	387.6	391.0
Total sperm count	$x\ 10^6$	337.2	463.4	963.9	1241.37	166.32	211.8	583.95	509.73	335.7	509.95	1106.3	1164.76	282.88	448.0	1395.36	1368.5
MOT	%	55	74	88	90	67	97	93	87	62	86	87	92	68	87	89	92
PMOT	%	36	62	80	85	28	78	74	81	50	77	81	86	41	79	75	87
VAP	µm/s	89.5	132.8	149.9	138.4	96.6	134.2	156.9	160.5	92.6	111.6	143.5	152.1	83.7	117.9	154.6	161.5
VSL	µm/s	87.9	127.9	132.4	131.8	90.3	112.2	145.0	142.4	87.9	100.6	132.3	139.7	81.3	113.8	133.2	139.2
VCL	µm/s	137.2	178.3	201.2	202.3	117.7	175.9	187.4	225.3	121.4	145.2	206.7	208.5	114.8	178.3	199.3	199.3
ALH	µm	5.2	7.2	6.2	6.3	2.6	6.3	6.4	7.6	2.2	5.3	7.5	7.8	3.4	7.3	6.9	6.9
BCF	Hz	28.9	30.6	29.8	31.6	24.7	20.2	27.3	29.1	21.3	29.6	33.4	33.7	19.4	24.1	32.4	31.2
STR	%	98	96	88	95	93	81	92	88	94	90	92	92	97	96	86	86
LIN	%	64	71	65	65	76	64	79	65	72	69	64	67	70	63	66	69
RAPID	%	30	58	78	79	29	84	86	86	47	69	80	80	37	70	70	81
Medium	%	14	10	7	8	37	13	6	6	14	15	11	12	11	15	18	9
Slow	%	21	9	5	8	27	3	5	6	18	7	4	4	28	9	6	7
STATIC	%	35	23	10	5	7	1	3	2	21	9	5	4	24	6	6	3

Table 3. The percentage of live and normal spermatozoa in four male dogs (I, II, III and IV) under supplementation with selenium and vitamin E on days 0, 30, 60 and 90

Parameter	Dog															
	I				II				III				IV			
	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90
Live spermatozoa (%) (eosin-nigrosin)	69	78	89	91	71	92	95	92	71	90	96	95	72	89	88	93
Normal spermatozoa (%)	62	75	89	87	52	71	79	81.5	61	78	88	84	71	87	91	89

Discussion

In most cases of infertility in dogs, the cause of poor semen quality remains unknown (12, 20). There are a variety of causative factors, such as hormonal disturbances, heat, stress, toxins, or autoimmune disorders (12). In the four cases described the possible cause of infertility was oxidative stress following selenium and vitamin E deficiency. This phenomenon, due to excess of reactive oxygen species (ROS) production, is an important cause of male infertility (2). Selenium is a constituent of the antioxidant enzyme GPx and together with vit. E plays an important role in the protection of spermatozoa against ROS damage. Selenium is indispensable to the process of production and maturation of spermatozoa (5, 25–27). Vitamin E is a well-documented antioxidant, inhibiting free radical-induced damage to sperm membranes. Its oral administration improves sperm motility and morphology (14, 27, 28). Both this and Se are usually added to commercial diets for dogs. Selenium levels vary in diets in step with ingredients used in pet food formulations (37). In commercial diets inorganic selenium compounds, sodium selenite, or sodium selenate are the selenium sources generally added. However, inorganic selenium is less bioavailable than the organic selenium compounds (18, 39). Vitamin E is added to pet food mainly as α-tocopherol; however, significant losses of this compound were found after a relatively short product storage time (8). The references values for Se and vit. E in blood serum were described in only a few papers. Pilarczyk

et al. (31) reported a Se range of 208–346 µg/L and van Zelst *et al*. (39) 213–310 µg/L for healthy dogs. The concentrations of vit. E varied from 11.2 to 41.1 mg/L. Jewell *et al*. (19) reported concentrations of vit. E of 27.30–36.74 mg/L for dogs.

There are still only few data about the influence of Se and vit. E on semen quality and fertility in dogs and the results are inconsistent. In humans and other species Se's positive effect on spermatozoa concentration was reported (21, 26, 27, 34, 35, 38). Contrary findings came from other studies showing no positive effect of Se supplementation on sperm quality in humans (15, 17) or boars (16).

Supplementation with vit. E significantly decreased the concentration of malondialdehyde (MDA), a biomarker of oxidative stress (29, 36). Vitamin E and Se administered together were more effective in raising semen quality in boars than vit. E alone (26). Our previous study showed that supplementation with Se and vit. E enhanced the antioxidant status of spermatozoa and improved semen quality in clinically healthy dogs with lowered fertility (9, 11).

In the described cases, a salient result was the rapid increase in sperm motility within 30 days of supplementation with Se and vit. E due to their effect on the existing reservoir of sperm cells. Concentration of spermatozoa improved more slowly, after 60 days, which related to the duration of the spermatogenesis process in dogs. The continuous improvements in motility parameters were also correlated with better spermatozoa morphology. The rise in percentage of normal and live sperm cells is correlated with the increase in selenoprotein P activity, which supplies selenium for spermatogenesis. In the absence of selenoprotein P an increase in the production of defective spermatozoa occurs (6, 23).

Several studies on other animal species and on humans also showed a beneficial effect of supplementation with Se and/or vit. E on semen quality (1, 2, 40). The preparation also contained 50 mg of evening primrose extract, which is a rich source of polyunsaturated fatty acids (3). They are the structural components of sperm cell membranes and serve as antioxidant defence (7).

These four cases described showed that supplementation with Se and vit. E in dogs with low Se status leads to the rapid improvement of semen motility after only one month followed by an increase in sperm concentration and restoration of fertility. Studies on a larger number of dogs and on individuals with different histories of infertility are still in process and will be the subject of further publications.

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