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

Anna Domosławska, Sławomir Zduńczyk, Tomasz Janowski

Department of Animal Reproduction with Clinic, Faculty of Veterinary Medicine, University of Warmia and Mazury, 10-719 Olsztyn, Poland
anna.domoslawska@gmail.com

Received: January 10, 2019 Accepted: April 29, 2019

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 etiology (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 × 10⁶ 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.

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 | Dog II | Dog III | Dog IV |
|-----------------|-------|--------|---------|--------|
|                 | Day 0 | Day 30 | Day 60  | Day 90 |
|                  |       |        |         |        |
| Selenium (µg/L) | 86    | 279    | 317     | 352    |
| Vitamin E (mg/L)| 11.5  | 29.7   | 42.1    | 40.1   |

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.

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 Nižański 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

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.),
- 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 × 10⁶ 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 Nižański 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 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 | 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 |
|-----------|------|-------|--------|--------|--------|-------|--------|--------|--------|-------|--------|--------|--------|-------|--------|--------|--------|
| Total sperm count | x10^6/mL | 337.2 | 463.4 | 963.9 | 1241.37 | 166.32 | 211.8 | 583.95 | 509.73 | 335.7 | 509.95 | 1164.76 | 228.88 | 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 |
| 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 |
| 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 |
| STR % | | 98 | 96 | 88 | 95 | 95 | 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 | Day 0 | Day 30 | Day 60 | Day 90 |
|-----------|-----|-------|--------|--------|--------|
| Live spermatozoa (%) (eosin-nigrosin) | I | 69 | 78 | 89 | 91 |
| II | 71 | 92 | 95 | 92 |
| III | 92 | 71 | 90 | 96 |
| IV | 95 | 72 | 89 | 88 |
| Normal spermatozoa (%) | I | 62 | 75 | 89 | 87 |
| II | 72 | 51 | 71 | 79 |
| III | 81.5 | 61 | 78 | 88 |
| IV | 84 | 71 | 87 | 91 |

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.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The sources of funding for the research and the article were the University of Warmia and Mazury and owners of animals which were patients of the Animal Reproduction Clinic in the Department of Animal Reproduction.

Animal Rights Statement: All animals were regular patients of the Animal Reproduction Clinic in the Department of Animal Reproduction, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn.

References
1. Ahsan U., Kamran Z., Raza I., Ahmad S., Babar S., Riaz M.H., Iqbal Z.: Role of selenium in male reproduction – a review. Anim Reprod Sci 2014, 146, 55–62.
2. Artken R.J., Smith T.B., Jobling M.S., Baker M.A., De Juliss G.N.: Oxidative stress and male reproductive health. Asian J Androl 2014, 16, 31–38.
3. Bayles B., Usatine R.: Evening primrose oil. Am Fam Physician 2009, 80, 1405–1408.
4. Blanco-Rodriguez J., Martinez-Garcia C.: In vivo analysis of germ cell apoptosis reveals the existence of a stage-specific “social” control on germ cell death in the seminiferous epithelium. Int J Androl 1997, 20, 373–378.
5. Boitani C., Puglisi R.: Selenium, a key element in spermatogenesis and male fertility. Adv Exp Med Biol 2008, 636, 65–73.
6. Burk R.F., Hill K.E.: Selenoprotein P expression, functions, and roles in mammals. Biochim Biophys Acta 2009, 1790, 1441–1447.
7. Da Rocha A.A., da Cunha I.C.N., Ederli B.B., Albernaz A.P., Quirino C.R.: Effect of daily food supplementation with essential fatty acids on canine semen quality. Reprod Dom Anim 2009, 44, Suppl 22, 313–315.
8. De Man J.: Principles of food chemistry. Aspen Publishers Inc., Gaithersburg, 1999, pp. 365–368.
9. Domosławska A., Zduńczyk S., Franczyk M., Janowski T., Kankofer M.: Selenium and vitamin E supplementation enhances the antioxidant status of spermatozoa and improves semen quality in male dogs with lowered fertility. Andrologia 2018, 50, e13023, Doi: 10.1111/and.13023.
10. Domosławska A., Zduńczyk S., Niżakski W., Janowski T.: Assessment of semen quality in infertile dogs using computer-assisted sperm analysis by the Hamilton-Thorne Semen Analyser. Bull Vet Inst Pulawy 2013, 57, 429–432.
11. Domosławska A., Zduńczyk S., Niżakski W., Jurczak A., Janowski T.: Effect of selenium and vitamin E supplementation on semen quality in dogs with lowered fertility. Bull Vet Inst Pulawy 2015, 59, 85–90.
12. Fontbonne A.: Infertility in male dogs: recent advances. Rev Bras Reprod Anim 2011, 35, 266–273.
13. Günzel-Apel A-R., Terhaar P., Waberski D.: Hodendimensionen und Ejakulatbeschaffenheit fertiler Rüden unterschiedlicher Körpergewichte. Kleintierpraxis 1994, 39, 483–486.
14. Hatamoto L.K., Sobrino B.C.A., Nichi N., Barnabe V.H., Barnabe R.C., Cortada C.N.M.: Effect of dexamethasone treatment (to mimic stress) and vitamin E oral supplementation on the spermogram and on seminal plasma spontaneous lipid peroxidation and antioxidant enzyme activities in dogs. Theriogenology 2006, 66, 1610–1644.
15. Hawkes W.C., Turek P.: Effects of dietary selenium on sperm motility in healthy men. J Androl 2001, 22, 764–772.
Henson M.C., Kattesh H.G., Hitchcock J.P., Kincaid S.A.: The effect of dietary selenium on growth and selected reproductive parameters in young boars. Anim Prod 1983, 37, 401–407.

Iwanier K., Zachara B.A.: Selenium supplementation enhances the element concentration in blood and seminal fluid but does not change the spermatozoal quality characteristics in subfertile men. J Androl 1995, 16, 441–447.

Jacob J.H., Khalil A.M., Maslat A.O.: In vitro cyto genetic testing of an organoselenium compound and its sulfur analogue in cultured rat bone marrow cells. J Carcinog 2004, 3, 5–13.

Jewel D.E, Toll P.W., Wedekind K.J., Zicker S.C.: Effect of increasing dietary antioxidants on concentration of vitamin E and total alkenals in serum of dogs and cats. Vet Therapeutics 2000, 1, 264–272.

Johnston S.D., Root-Kustritz M.V., Olson P.N.S.: Canine and feline theriogenology. W.B. Saunders, Philadelphia 2001, pp. 370–388.

Kolodziej A., Jacyno E.: Effect of selenium and vitamin E supplementation on reproductive performance of young boars. Arch Tierz 2005, 48, 68–75.

Koziorowska-Gilun M., Strzeżek R.: Molecular forms of selected antioxidant enzymes in dog semen – electrophoretical identification. Pol J Vet Sci 2011, 14, 29–33.

Król M.B., Gromadzińska J., Wąsowicz W.: SeP, ApoER2, and megalin as necessary factors to maintain Se homeostasis in mammals. J Trace Elem Med Biol 2012, 26, 161–266.

Linde-Forsberg C.: Achieving canine pregnancy using frozen and chilled extended semen. Vet Clin North Am Small Anim Pract 2001, 21, 467–485.

Lubarda-Bierkowska Z., Majewska A.: Effect of selenium and low-molecular antioxidants on the quality of semen of farm animals. Post Nauk Rol 2010, 4, 81–90.

Marin-Guzman J., Mahan D.C., Chung Y.K., Pate J.L., Pope W.F.: Effects of dietary selenium and vitamin E on boar performance and tissue responses, semen quality, and subsequent fertilization rates in mature gilts. J Anim Sci 1997, 75, 2994-3003.

Martin-Guzman J., Mahan D.C., Whitmoyer R.: Effect of dietary selenium and vitamin E on the ultrastructure and ATP concentration of boar spermatozoa, and the efficacy of added sodium selenite in extended semen on sperm motility. J Anim Sci 2012, 78, 1544–1550.

Moslemi M.K., Tavanbakhash S.: Selenium-vitamin E supplementation in infertile men: effect on semen parameters and pregnancy rate. Int J Gen Med 2011, 4, 99–104.

Nielsen F., Mikkelsen B.B., Nielsen J.B., Andersen H.R., Grandjean P.: Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of lifestyle factors. Clin Chim 1997, 43, 1209–1214.

Nizâlski W., Partyka A., Ochota M., Antończyk A., Mikołajewska N., Błasiak K., Miła H., Stańczyk E.: Flow cytometric, computer assisted and traditional sperm analysis in fertile and subfertile dogs. Proc. 14th EVSSAR Congress, Milano 2011, p. 52.

Pilarczyk B., Tomza-Marciniak A., Pilarczyk R., Bąkowski M., Gail M., Wilk M., Kuba J.: Relationship between serum Se concentration in dogs and incidence of some disease conditions. Cent Europ Biol 2013, 8, 527–533.

Rijselaerz T., Maes D., Hoflack G., de Kruif A., Van Soom A.: Effect of body weight, age, and breeding history on canine sperm quality parameters measured by the Hamilton-Thorne analyser. Reprod Dom Anim 2007, 42, 143–148.

Simcock S.E., Rutherford S.M., Wester T.J., Hendriks W.H.: Total selenium concentrations in canine and feline foods commercially available in New Zealand. N Z Vet J 2005, 53, 1–5.

Slowińska M., Jankowski J., Dietrich G.J., Karol H., Liszewska E., Kozłowski K., Saratowska K., Ciereszko A.: Effect of organic and inorganic forms of selenium in diets on turkey semen quality. Poultry Sci 2011, 90, 181–190.

Speight S.M., Estienne M.J., Harper A.F., Crawford R.J., Knight J.W., Whitaker B.D.: Effects of dietary supplementation with an organic source of selenium on characteristics of semen quality and in vitro fertility in boars. J Anim Sci 2012, 90, 761–770.

Suleiman S.A., Ali M.E., Zauu Z.M.S., El-Malik E.M.A., Nasr M.A.: Lipid peroxidation and human sperm motility: protective role of vitamin E. J Androl 1996, 17, 530–537.

Turner T.T., Lysiak J.: Oxidative stress: a common factor in testicular dysfunction. J Androl 2008, 29, 488–498.

Udala J., Ramisz A., Drewnowski W., Lasota B., Radosz W.: The semen quality of bulls after application of selenium and vitamin E. Zesz Nauk AR Szczecin 1995, 168, 57–63.

Van Zelst M., Hesta M., Gray K., Beech K., Cools A., Alexander L.G., Du Lai G., Janssens G.P.: Selenium digestibility and bioactivity in dogs: what the can can, the kibble can't. PLoS One, 2016, 11 (4):e0152709. doi:10.1371/journal.pone.0152709.

Zubair M.: Effects of dietary vitamin E on male reproductive system. Asian Pac J Reprod 2017, 6, 145–150.