

Effect of selenium and vitamin E supplementation on semen quality in dogs with lowered fertility

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Abstract

Thirty clinically healthy dogs with poor semen quality were used in the study. Fifteen dogs were supplemented daily with selenium (0.6 mg/kg organic selenium from yeast) and vitamin E (5 mg/kg) *per os* for 60 d. The control group (15 dogs) was not supplemented. Semen was collected from all dogs by manual manipulation on days 0, 30, 60, and 90. The sperm concentration and motility parameters were evaluated with a Hamilton Thorne sperm analyser, version IVOS 12.3. For the assessment of sperm morphology, Diff-Quik stain was used. The percentage of live and dead spermatozoa was estimated on dried smears stained with eosin-nigrosin. The concentration of spermatozoa, most motility parameters determined (PMOT, VSL, VCL, ALH, BCF, RAPID, MEDIUM, SLOW, and STATIC), and the percentage of spermatozoa morphologically normal and live increased significantly ($P < 0.05$) after 60 d of supplementation. In the control group, there were no changes in motility parameters while the concentration and total sperm count decreased over the duration of the study. In conclusion, supplementation with selenium and vitamin E for 60 d can improve the quality of semen in dogs with lowered fertility.

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

Introduction

There is still not enough knowledge about infertility in the male dog. It can result from several problems including poor semen quality (6, 8, 10, 25).

Little attention is paid to proper diet for dogs that are in their reproductive years. Deficiency in nutrition may have a significant impact on the quality of the ejaculate. Among the requisite nutrients, selenium is one of the most important, which is essential for spermatogenesis (2, 22). Selenium occurs in the mammalian organism in the form of selenoproteins, which contain selenium as selenocysteine. Selenoprotein P is required for selenium supply to the testes (3, 20). An antioxidant enzyme – glutathione peroxidase (GPx) – is the major selenoprotein in the testes, which represents the link between selenium, sperm quality, and fertility (2). GPx is present in canine

spermatozoa and seminal plasma and plays the main role in the decomposition of hydrogen peroxide (19, 35). Studies on humans (26, 32), boars (18, 23, 24, 34), bulls (37), mice (39), and turkeys (33) demonstrated an evident connection between deficiency in selenium and fertility.

Vitamin E plays a key role in protecting spermatozoa against lipid peroxidation. Vitamin E supplementation showed a beneficial effect on semen quality in humans (36), boars (23, 24), and dogs (11). The two antioxidants, selenium and vitamin E, act synergistically and should be administered jointly (18, 22, 23, 24).

There is a significant interest in dietary supplements aimed at improving semen quality in humans and animals; however, there are few studies on canine semen quality after oral supplementation with vitamin E alone (11) and in combination with essential

fatty acids (5). The influence of selenium oral supplementation on the seminal parameters in the dog has not been investigated yet.

The aim of the study was to evaluate the effect of selenium and vitamin E supplementation on semen quality in male dogs with lowered fertility.

Material and Methods

The study was performed on 30 male dogs of various breeds. All dogs were referred to our Department of Animals Reproduction with Clinic department because of either conception failure within their last 2-3 matings with different bitches or a low number of puppies delivered after mating, compared to previous litters and typical litter size for the breed. The age of the dogs ranged from 3 to 8 years. All males were in good general condition with normal sexual libido without any food changes, any diseases with raised body temperature, or any drugs administered. The prostate gland and testes were examined by ultrasonography (8.0MHz probe, MyLab30Gold, Esaote, Italy).

The dogs in the experimental group ($n = 15$) were supplemented daily by 6 $\mu\text{g/kg}$ of organic selenium from yeasts and 5 mg/kg of vitamin E (Semevet, VetExpert®, Poland). Both compounds were administered *per os* for 60 d. The preparation also contained 50 mg of evening primrose extract. The control group, without supplementation, consisted of 15 males which corresponded to the experimental group of dogs in terms of age, body weight, and reproduction season of semen collection.

Semen was collected by manual manipulation as described by Linde-Forsberg (21) 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 tubes.

The sperm concentration and motility parameters were assessed with a Hamilton Thorne sperm analyser (USA), version IVOS 12.3 (HTR-IVOS 12.3). The software settings are shown in Table 1 (28).

Each ejaculate was 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), the velocity average pathway (VAP), the velocity straight line (VSL), the velocity curvilinear (VCL), the amplitude lateral head (ALH), the beat cross frequency (BCF), the straightness (STR), the linearity (LIN), and the motility subcategories RAPID, MEDIUM, SLOW, and STATIC.

The percentage of live and dead spermatozoa was estimated on dried smears stained with eosin-nigrosin. A monochromatic Diff-Quik stain was used for assessing sperm morphology. Two hundred spermatozoa were evaluated per slide, representing 100%.

The results were presented as mean and standard deviation. The sperm quality parameters were compared between both groups using a Mann–Whitney *U* test or Student's *t*-test (GraphPAD PRISM, Version 4.00, GraphPad Software, USA). The level of significance was set at $P < 0.05$.

Results

All dogs were clinically healthy without any diseases or abnormalities of the genital tract. Ultrasonography of the prostate gland and testes did not show any pathological conditions.

The mean values of semen quality parameters on consecutive days of examination are shown in Table 2.

Table 1. HTR-IVOS 12.3 software settings used in this study

Parameter	Value
Chamber type	Leja 4
Temperature of analysis (°C)	38.0
Fields acquired	10
Frame rate (Hz)	60
Minimum contrast	75
Minimum cell size (pixels)	6
Straightness (STR), threshold (%)	75
VAP cut-off ($\mu\text{m/s}$)	9.9
Prog. min VAP ($\mu\text{m/s}$)	100.0
VSL cut-off ($\mu\text{m/s}$)	20.0

Table 2. Volume, concentration, and motility parameters of semen (mean $\pm$ SD) in male dogs (n = 15) supplemented with selenium and vitamin E and in the control group (n = 15) on days 0, 30, 60, and 90

Parameter	Unit	Day 0		Day 30		Day 60		Day 90	
		experimental group	control group	experimental group	control group	experimental group	control group	experimental group	control group
Volume of the ejaculate (sperm-rich fraction)	mL	2.90 $\pm$ 1.29	2.26 $\pm$ 1.23	2.72 $\pm$ 0.98	2.08 $\pm$ 0.95	2.89 $\pm$ 1.12	2.31 $\pm$ 1.19	2.94 $\pm$ 1.05	2.36 $\pm$ 1.01
Concentration	$\times 10^6$ /mL	80.56 $\pm$ 47.98 ^a	114.76 $\pm$ 81.47 ^a	100.54 $\pm$ 44.42 ^c	101.11 $\pm$ 55.44	170.78 $\pm$ 76.65 ^{bde}	76.42 $\pm$ 32.80 ^{bf}	186.82 $\pm$ 62.25 ^{bde}	77.66 $\pm$ 45.76 ^{bf}
Total sperm count	$\times 10^6$	253.24 $\pm$ 217.3 ^a	278.15 $\pm$ 233.08 ^a	283.99 $\pm$ 180.38 ^c	211.61 $\pm$ 137.34	502.51 $\pm$ 329.61 ^{bde}	183.09 $\pm$ 125.25 ^{bf}	560.05 $\pm$ 305.25 ^{bde}	192.97 $\pm$ 130.66 ^{bf}
MOT	%	73.93 $\pm$ 15.01	66.07 $\pm$ 21.65	76.86 $\pm$ 12.67	64.46 $\pm$ 30.89	75.80 $\pm$ 10.55	64.30 $\pm$ 29.23	78.00 $\pm$ 8.69 ^e	58.53 $\pm$ 26.79 ^f
PMOT	μ m/s	41.53 $\pm$ 20.41 ^a	34.93 $\pm$ 20.32	51.53 $\pm$ 14.93 ^e	36.84 $\pm$ 23.17 ^f	55.60 $\pm$ 0.47 ^{be}	34.38 $\pm$ 20.84 ^f	63.53 $\pm$ 8.24 ^{be}	32.00 $\pm$ 23.32 ^f
VAP	μ m/s	129.74 $\pm$ 31.76	125.81 $\pm$ 25.11	137.80 $\pm$ 21.67	132.03 $\pm$ 21.98	144.45 $\pm$ 14.35	124.81 $\pm$ 14.81	144.68 $\pm$ 13.81	129.45 $\pm$ 26.49
VSL	μ m/s	112.53 $\pm$ 28.71 ^a	106.72 $\pm$ 41.96	127.14 $\pm$ 16.85	108.46 $\pm$ 48.43	136.54 $\pm$ 13.33 ^{be}	108.74 $\pm$ 40.46 ^f	128.14 $\pm$ 17.64	115.52 $\pm$ 36.01
VCL	μ m/s	175.45 $\pm$ 45.78 ^a	167.76 $\pm$ 18.59	192.28 $\pm$ 32.40	178.37 $\pm$ 19.62	198.63 $\pm$ 28.30	173.54 $\pm$ 22.52	205.16 $\pm$ 24.85 ^{be}	176.91 $\pm$ 18.30 ^f
ALH	μ m	4.39 $\pm$ 1.16 ^a	4.88 $\pm$ 2.17	4.70 $\pm$ 0.91	5.33 $\pm$ 1.63	4.81 $\pm$ 0.81	4.91 $\pm$ 1.79	5.04 $\pm$ 0.74 ^b	5.02 $\pm$ 1.35
BCF	Hz	17.18 $\pm$ 7.37 ^a	22.54 $\pm$ 2.92	21.14 $\pm$ 3.62 ^c	22.37 $\pm$ 5.14	25.58 $\pm$ 3.64 ^b	24.71 $\pm$ 3.98	27.55 $\pm$ 4.05 ^{bd}	26.26 $\pm$ 4.05
STR	%	87.40 $\pm$ 4.12	86.24 $\pm$ 18.05	87.26 $\pm$ 3.71	87.11 $\pm$ 19.56	86.80 $\pm$ 4.19	86.02 $\pm$ 28.78	87.20 $\pm$ 4.98	88.24 $\pm$ 16.18
LIN	%	65.73 $\pm$ 8.12	65.77 $\pm$ 19.99	65.66 $\pm$ 6.27	64.98 $\pm$ 18.18	67.26 $\pm$ 6.11	62.48 $\pm$ 21.46	66.00 $\pm$ 6.18	65.31 $\pm$ 18.19
RAPID	%	47.00 $\pm$ 19.16 ^a	43.38 $\pm$ 26.80	54.53 $\pm$ 14.83	48.23 $\pm$ 25.37	62.80 $\pm$ 9.69 ^{be}	43.00 $\pm$ 25.29 ^f	65.93 $\pm$ 8.50 ^{be}	44.07 $\pm$ 25.55 ^f
MEDIUM	%	24.00 $\pm$ 6.02 ^a	21.00 $\pm$ 12.88	25.73 $\pm$ 6.85 ^c	21.84 $\pm$ 14.34	19.06 $\pm$ 6.28 ^{de}	25.53 $\pm$ 16.45 ^f	17.26 $\pm$ 5.88 ^{bde}	25.84 $\pm$ 16.32 ^f
SLOW	%	15.66 $\pm$ 8.44 ^{ae}	21.30 $\pm$ 16.06 ^f	12.33 $\pm$ 6.84	16.53 $\pm$ 13.61	10.86 $\pm$ 4.40 ^e	17.38 $\pm$ 16.84	9.66 $\pm$ 3.49 ^{be}	17.76 $\pm$ 12.08
STATIC	%	12.66 $\pm$ 10.95 ^a	14.23 $\pm$ 9.83	8.00 $\pm$ 6.94 ^e	13.30 $\pm$ 8.44 ^f	7.26 $\pm$ 3.05 ^{be}	14.46 $\pm$ 12.75 ^f	7.13 $\pm$ 2.78 ^{be}	12.46 $\pm$ 8.96 ^f

The volume of the sperm-rich fraction was not significantly differ between different days of sperm collection, between groups, or over the duration of the study. Concentration and total cell count of spermatozoa increased significantly ($P < 0.05$) after 60 d in the supplemented group and decreased in the control group (Table 2).

Most motility parameters determined (PMOT, VSL, VCL, ALH, BCF, RAPID, MEDIUM, SLOW,

and STATIC) increased significantly ($P < 0.05$) after one month of supplementation while they did not change in the control group (Table 2). There were no significant differences ($P < 0.05$) for MOT (Table 2), STR, or LIN (Table 1) over the duration of the study in the supplemented group or the control group separately but there were distinct differences between these groups for MOT on day 90 of the study (Table 2).

Table 3. The percentage of live, normal, and abnormal spermatozoa and spermatozoa with major and minor defects (mean $\pm$ SD) in male dog (n = 15) supplemented with selenium und vitamin E and in the control group (n = 15) on days 0, 30, 60, and 90

Parameter	Day 0		Day 30		Day 60		Day 90	
	experimental group	control group	experimental group	control group	experimental group	control group	experimental group	control group
Live spermatozoa (%) (eosin-nigrosin)	80.26 $\pm$ 11.65 ^a	71.00 $\pm$ 13.72 ^a	85.20 $\pm$ 8.05 ^{ce}	61.69 $\pm$ 27.67 ^f	91.93 $\pm$ 4.07 ^{bde}	61.57 $\pm$ 30.71 ^{bf}	93.06 $\pm$ 3.08 ^{bde}	59.08 $\pm$ 29.42 ^{bf}
Normal spermatozoa (%)	70.13 $\pm$ 21.72 ^a	73.62 $\pm$ 13.64 ^a	74.13 $\pm$ 12.84	66.29 $\pm$ 11.24	80.86 $\pm$ 7.35 ^e	67.00 $\pm$ 12.17 ^f	83.93 $\pm$ 6.14 ^{be}	65.05 $\pm$ 12.68 ^{bf}
Total abnormal spermatozoa (%)	29.87 $\pm$ 21.72 ^a	26.37 $\pm$ 13.64 ^a	25.87 $\pm$ 12.84	33.70 $\pm$ 11.24	19.14 $\pm$ 7.35 ^e	33.00 $\pm$ 12.17 ^f	16.07 $\pm$ 6.14 ^{be}	34.95 $\pm$ 12.68 ^{bf}
Major defects (%)	8.5 $\pm$ 6.46	12.00 $\pm$ 4.22 ^a	10.83 $\pm$ 6.19 ^e	20.37 $\pm$ 9.29 ^{bf}	8.20 $\pm$ 3.83 ^e	17.66 $\pm$ 7.33 ^{bf}	7.26 $\pm$ 2.78 ^e	19.70 $\pm$ 8.72 ^{bf}
Minor defects (%)	21.37 $\pm$ 17.05 ^{ae}	14.37 $\pm$ 10.52 ^f	15.04 $\pm$ 10.69 ^c	13.33 $\pm$ 5.57	10.94 $\pm$ 4.68 ^b	15.33 $\pm$ 8.03	8.81 $\pm$ 4.55 ^{bde}	15.25 $\pm$ 6.91 ^f

Means with a b and c d – pairs of statistical differences within the row separately for supplemented and control group; means with e f – pairs of statistical differences within the row between supplemented and control group in each day of the study

In the experimental group, the percentage of live spermatozoa increased significantly ($P < 0.05$), while the percentage of abnormal spermatozoa and spermatozoa with minor morphological changes significantly decreased ($P < 0.05$) over 60 d (Table 3). In the control group, live and normal spermatozoa significantly ($P < 0.05$) decreased in the same period and the increase in major defects of spermatozoa was observed.

Discussion

In the study, the sperm concentration, percentage of spermatozoa with normal morphology, and most evaluated motility parameters (except for MOT, VAP, STR, and LIN) of dogs with lowered fertility were poorer than reported for fertile dogs (6, 14, 17, 29, 30). These dogs with lowered fertility were clinically healthy and the cause of poor semen quality could not be diagnosed. Testicular degeneration was assumed as the possible cause of the poor semen quality in these dogs, which is a common phenomenon. A variety of causative factors, such as hormonal disturbances, heat stress, toxins, and autoimmune disorders have been reported (8, 10, 25).

The results obtained in this study showed that supplementation with selenium and vitamin E for 60 d can improve the quality of semen in dogs with lowered fertility. Similar results were reported for humans (16,

25), boars (18, 23, 24, 34), and turkeys (33) after supplementation with selenium and vitamin E. To our knowledge, no studies have been carried out on the influence of oral selenium supplementation on semen quality in the dog.

Selenium is essential for the process of production and maturation of spermatozoa (2, 22, 23, 24). The function of selenium is mediated by selenoproteins, which are structural components of the sperm midpiece (2, 3, 20). Selenium has a positive effect (18, 23) on the activity of the antioxidant enzyme - glutathione peroxidase, which has been found in dog semen (19, 35). Therefore a higher resistance to oxidative stress should be expected when selenium is provided. A positive effect of selenium on sperm concentration and morphology has been also reported by other authors in men (26, 32) and various animal species (18, 23, 24, 33, 34, 37). On the contrary, some studies have reported that there were no positive effects of selenium supplementation on sperm quality either in humans (12, 15) or boars (13).

Most of the motility parameters in the experimental group increased during the study, however, there was no statistical difference in MOT, except in five males, in which the motility was approximately 60% of the value or less. In all dogs with lowered fertility, the problem was not motility intrinsically, but a low percentage of spermatozoa with progressive motility. The ability to fertilise seems to be strictly connected with progressive motility, which is

dependent on velocity parameters (VAP, VSL, and VCL) and also on ALH and BCF. These values define movement of spermatozoa and its ability to pass through cervical mucus and penetrate the zona pelucida (7, 31). This was reported in humans where VCL and BCF were higher for fertile men (9). In dogs under supplementation, these parameters increased to values typical of their fertile counterparts (6, 14, 17, 29, 30). Additionally, there were found changes in speed of spermatozoa movement, an increase in the rapid spermatozoa population, and a decrease in the slow and static cell population. In contrast to the experimental group, there were no changes in the main motility parameters (MOT, PMOT, VAP, VSL, VCL, BCF, ALH, RAPID, MEDIUM, SLOW, and STATIC) in the control group. The concentration and total sperm count in the control dogs decreased during the study. This indicates a progressive impairment of spermatogenesis in these dogs.

An increase in motility parameters in the experimental group also correlated with better semen morphology. There was an increase in the percentage of normal and live spermatozoa, which is in turn linked to the activity of selenoprotein P. The majority of plasma selenium is determined by this selenoprotein, which supplies selenium for spermatogenesis and which, if absent, blocks selenium supply and leads to defective spermatozoa production (3, 20). In a mouse model with low selenium diet, the reversion of a high number of flagellar structure defects in spermatozoa and restoration of fertility by a normal selenium diet were demonstrated (2, 38). These observations are similar to the results of our study, where under supplementation a lower number of defects of sperm flagellum was found. In counterpoint to this, a decrease in live spermatozoa and increase in total abnormal sperm cells were observed in the group without supplementation.

There are only few data regarding the effect of vitamin E supplementation on the seminal parameters in the dog. Hatamoto *et al.* (11) reported that supplementation with vitamin E increased sperm motility, vigour, and concentration, decreased the percentage of major sperm defects and overcame the negative effect on semen quality induced by dexamethasone treatment.

Vitamin E is a well-documented antioxidant, which inhibits free radical-induced damage to sperm cell membranes, and its oral administration led to improvement of sperm motility and morphology (11, 24, 26). Supplementation with vitamin E significantly decreased the concentration of malondialdehyde (MDA), a biomarker for oxidative stress (27, 36). Vitamin E together with selenium was more effective in raising semen quality in boars than vitamin E alone (23).

The preparation used also contained 50 mg of evening primrose extract, a rich source for polyunsaturated fatty acids (1), that are the structural

components of spermatozoon cell membranes and have an antioxidant effect. It was shown that supplementation with essential fatty acid together with vitamin E significantly improved the quality of canine ejaculate (5).

Although selenium and vitamin E are important nutrients for good quality of semen, it should be mentioned that their excess can cause spermatozoa damage. High doses of selenium in the diet caused a decrease in sperm motility in men (12). Vitamin E at a dose 20 times higher than the daily requirement exerted a deleterious effect on the sperm cells of cocks (4).

In conclusion, oral supplementation with selenium and vitamin E for 60 d significantly increased concentration, motility, and morphological parameters of spermatozoa in clinically healthy dogs with lowered fertility.

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References

1. Bayles B., Usatine R.: Evening primrose oil. *Am Fam Physician* 2009, 80, 1405–1408.
2. Boitani C., Puglisi R.: Selenium, a key element in spermatogenesis and male fertility. *Adv Exp Med Biol* 2008, 636, 65–73.
3. Burk R.F., Hill K.E.: Selenoprotein P—expression, functions, and roles in mammals. *Biochim Biophys Acta* 2009, 1790, 1441–1447.
4. Danikowski S., Sallmann H.P., Halle I., Flachowsky G.: Influence of high levels of vitamin E on semen parameters of cocks. *J Anim Physiol Anim Nutr* 2002, 86, 376–382.
5. Da Rocha A.A., da Cunha I.C.N., Ederli B.B., Albermaz 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.
6. Domoslawska A., Zdunczyk S., Nizanski 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.
7. Donnelly E.D., Lewis S.E.M., McNally J.A., Thompson W.: *In vivo* fertilization and pregnancy rates: the influence of sperm motility and morphology on IVF outcome. *Fert Steril* 1998, 70, 304–314.
8. Feldmann J., Nelson R.W.: Canine and feline endocrinology and reproduction. W.B. Saunders, Philadelphia, 1996, pp. 718–733.
9. Fetterlof P.M., Rogers B.J.: Prediction of human sperm penetrating ability using computerized motion parameters. *Mol Reprod Dev* 1990, 27, 326–331.
10. Fontbonne A.: Infertility in male dogs: recent advances. *Rev Bras Reprod Anim* 2011, 35, 266–273.
11. Hatamoto L.K., Sobrinho 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 spermiogram and on seminal plasma spontaneous lipid peroxidation and antioxidant enzyme activities in dogs. *Theriogenology* 2006, 66, 1610–1644.
12. Hawkes W.C., Turek P.: Effects of dietary selenium on sperm motility in healthy men. *J Androl* 2001, Vol 22 (5), 764–772.
 13. 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.
 14. Iguer-Ouada M., Verstegen J.: Evaluation of the Hamilton Thorn computer-based automated system for dog semen analysis. *Theriogenology* 2001, 55, 733–749.
 15. 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.
 16. Keskes-Anmar L., Feki-Chakroun N., Rebai T., Sahnoun Z., Ghazzi H., Hammami S., Zghal K., Feki H., Damak J., Bahloul A.: Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl* 2002, 49, 83–94.
 17. Klimowicz M., Nizanski W., Savic M., Zbyryt I., Dubiel A.: Evaluation of dog semen quality using a conventional microscopic method, flow cytometry and HTM IVOS computer sperm quality analyzer. *Med Weter* 2005, 61, 1250–1255.
 18. Kołodziej A., Jacyno E.: Effect of selenium and vitamin E supplementation on reproductive performance of young boars. *Arch Tierz* 2005, 48, 68–75.
 19. Koziorowska-Gilun M., Strzeżek R.: Molecular forms of selected antioxidant enzymes in dog semen - electrophoretic identification. *Pol J Vet Sci* 2011, 14, 29–33.
 20. Krol M.B., Gromadzinska J., Wasowicz W.: SeP, ApoER2 and megalin as necessary factors to maintain Se homeostasis in mammals. *J Trace Elem Med Biol* 2012, 26, 161–266.
 21. Linde-Forsberg C.: Achieving canine pregnancy using frozen and chilled extended semen. *Vet Clin North Am Small Anim Pract* 2001, 21, 467–485.
 22. Lubierda-Bieńkowska Z., Majewska A.: Wpływ selenu i niskocząsteczkowych antyoksydantów na jakość nasienia zwierząt gospodarskich. *Post Nauk Rol* 2010, 4, 81–90.
 23. 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.
 24. Marin-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.
 25. Mickelsen W.D., Memon M.A., Anderson P.B., Freeman D.A.: The relationship of semen quality to pregnancy rate and litter size following artificial insemination in the bitch. *Theriogenology* 1993, 39, 553–560.
 26. Moslemi M.K., Tavanbakhsh S.: Selenium–vitamin E supplementation in infertile men: effect on semen parameters and pregnancy rate. *Int J Gen Med* 2011, 4, 99–104.
 27. 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 life–style factors. *Clin Chem* 1997, 43, 1209–1214.
 28. Nizański W., Klimowicz M., Partka A., Savic M., Dubiel A.: Effects of inclusion of Equex STM into tris–based extender on the motility of dog spermatozoa incubated at 5 degrees C. *Reprod Dom Anim* 2009, 44, 363–365.
 29. Nizański 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.
 30. Rijselaere 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.
 31. Sallam H.N., Ezzeldin F., Sallam A., Agameya A.F., Farrag A.: Sperm velocity and morphology, female characteristics, and the hypo-osmotic swelling test as predictors of fertilization potential: experience from the IVF model. *Int J Fertil Womens Med* 2003, 48, 88–95.
 32. Scott R., MacPherson A., Yates R.W., Hussain B., Dixon J.: The effect of oral selenium supplementation on human sperm motility. *Br J Urol* 1998, 82, 76–80.
 33. Słowińska 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.
 34. 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, 761–70.
 35. Strzeżek R., Koziorowska-Gilun M., Kowalówka M., Strzeżek J.: Characteristics of antioxidant system in dog semen. *Pol J Vet Sci* 2009, 12, 55–60.
 36. Suleiman S.A., Ali M.E., Zaiu 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.
 37. Udała J., Ramisz A., Drewnowski W., Lasota B., Radoch W.: The semen quality of bulls after application of selenium and vitamin E. *Zesz Nauk AR Szczecin* 1995, 168, 57–63.
 38. Verstegen J., Iguer-Ouada M., Onclin M.: Computer assisted semen analyzers in andrology research and veterinary practice. *Theriogenology* 2002, 57, 149–179.
 39. Watanabe T., Endo A.: Effect of selenium deficiency on sperm morphology and spermatocyte chromosomes in mice. *Mutat Res* 1991, 262, 93–99.