Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2021.18484.1646

Effect of Ginger Extract on Semen Parameters and Sperm DNA Fragmentation during the Liquid Storage at 4°C in Native Roosters (Research Note)

Sharafi M[1], Mohammadzadeh S[1], Mousavi SM[1] & Mumivand H[2]

[1] Department of Animal Science, Faculty of Agriculture, Lorestan University, Khorram Abad, Iran
[2] Department of Horticulture, Faculty of Agriculture, Lorestan University, Khorram Abad, Iran

Poultry Science Journal 2021, 9(1): 1-6

Keywords
Rooster
Antioxidant
Ginger extract
DNA fragmentation

Corresponding author
Saied Mohammadzadeh
mohammadzadeh.s@gmail.com

Article history
Received: October 22, 2020
Revised: April 12, 2021
Accepted: April 29, 2021

Abstract
The goal of the current study was to determine the effect of ginger extract on some quality parameters of rooster spermatozoa during liquid storage. A total of 10 healthy adult native roosters were selected and sampled twice a week for three weeks. Treatment groups were made up of basal extender supplemented with various concentrations of ginger extract including 0, 20, 40, 60, and 80 µg/mL. Sperm plasma membrane integrity, viability, and motility were assessed at 0, 24, 48, and 72 hours after semen collection. However, the sperm DNA fragmentation test was determined only after 48 hours of storage. The sperm membrane integrity was high at 24 (81.66%) and 48 (58.66%) hours in control and 60 µg/mL of ginger extract, respectively. The highest sperm viability (70.50%) and motility (76.50%) were obtained in 20 µg/mL ginger extract at 48h. The lowest sperm DNA fragmentation (32%) was observed in the 20 µg/mL of ginger extract. According to the results, the ginger extract showed a protective effect against time-associated harmful changes in semen quality of roosters during liquid storage.

Introduction

Avian sperm contains an antioxidant system that includes non-enzymatic antioxidants such as vitamins E and C, and also enzymatic antioxidants such as glutathione peroxidase and superoxide dismutase (Surai *et al*., 1998). Sustaining a balance between reactive oxygen species (ROS) and the antioxidant system is essential, which interferes with semen preservation in liquid or freezing conditions. Poultry sperm is more sensitive to lipid peroxidation due to the high concentration of unsaturated fatty acids (PUFAs). Sperm storage at low temperatures increases the production of ROS (Poorrostami and Farokhi, 2016). ROS has detrimental effects on sperm structure, and these effects are not only limited to membrane changes but also cellular metabolism. In other words, lipid peroxidation disrupts mitochondrial function and enhances DNA fragmentation (Sanocka and Kurpisz, 2004).

Although the beneficial effects of synthetic antioxidants on semen quality have been shown previously by Akhlaghi *et al*. (2014), the use of natural antioxidants has attracted more attention because synthetic antioxidants have toxic and carcinogenic effects (Yanishlieva and Marinova, 1996). Considering natural antioxidants, flavonoid compounds inhibit oxidation and disintegration of cell membrane phospholipids (Bode & Dong, 2011). Ginger (*Zingiber officinale*) is a flowering plant whose rhizome called ginger root, or simply ginger, has high antioxidant compounds and is used in fresh, dried, pickled, preserved, crystallized, extracted, and powdered forms. The ginger extract contains terpenes, alcohols, ketones, flavonoids, and phytoestrogens substance that has medicinal uses (Meutia., 2018). Ginger has been fractionated into at least 14 bioactive compounds, including [4]-gingerol, [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-paradol, [14]-shogaol, [6]-shogaol, 1-dehydro-[10]-gingerdione, [10]-gingerdione, hexahydrocurcumin, tetrahydrocurcumin, gingerenone A, 1,7-bis-(4′ hydroxyl-3′ methoxyphenyl)-5-methoxyhepthan-3-one, and methoxy-[10]-gingerol (Bode & Dong, 2011). Ginger extract eliminated free radicals during spermatogenesis and increased libido in rats (Altman and Marcussen, 2001; Khaki *et al*., 2009). Dietary

Please cite this article as Sharafi M, Mohammadzadeh S, Mousavi SM & Mumivand H. 2021. Effect of Ginger Extract on Semen Parameters and Sperm DNA Fragmentation during the Liquid Storage at 4°C in Native Roosters (Research Note). Poult. Sci. J. 9(1): 1-6.

supplementation of ginger powder showed an improvement of sperm motility, viability, and plasma membrane integrity in 52-week-old Cobb roosters (Akhlaghi *et al*., 2014). Further, administration of ginger powder in the rat diet significantly increased sperm viability, motility, and serum total testosterone concentrations (Khaki *et al*., 2009).

Slameňová *et al*. (2002) reported that sperm DNA damage is a valuable biomarker indicating the functionality of sperm cells. Compared to high motile sperms, a high percentage of low motile sperms show DNA fragmentation (Banks *et al.*, 2005).To the best of our knowledge, there is no information about the effect of ginger extract on the nuclear status of rooster sperms particularly during in vitro storage. Therefore, the objective of this study was to assess the effect of different levels of ginger extract on rooster sperm motility, viability, plasma membrane integrity, and DNA fragmentation during liquid storage.

Materials and Methods

Preparation of rosemary aqueous extract

For the preparation of the ginger aqueous extract, the ginger rhizome (400 g) was crushed and dried. The powder was weighed and mixed with 80% of ethanol (1:9 W/V) and allowed to soak for three days at room temperature. The mixture was thoroughly mixed and filtered to remove the debris. The solution was distilled in a rotary (at 78°C) to extract the ethanol. The extract was then stored in dark conditions at 4°C (Garcia-Salas *et al.,* 2010). The stock solution (10x) was prepared using a sexton extender. Ginger extract was dissolved in dimethyl sulfoxide (DMSO) and different concentrations of 20, 40, 60, and 80 µg/mL were prepared.

Semen collection and analysis

The experiment was performed using 10 native 52-week-old roosters (Gallus gallus *domesticus*). The roosters were kept in individual cages of 70 × 70 × 85 cm at a temperature of 24°C under a standard light program (14 h of light and 10 h of dark), water and feed were provided *ad-libitum*. Dietary energy and protein were 3170 kcal/kg and 12%, respectively.

The roosters were subjected to biweekly semen collections for 3 weeks using the abdominal massage method. Immediately after collection in microtube, the ejaculates were transferred to a water bath at 37°C and then evaluated. Only ejaculates with the $>3 \times 10^9$ spermatozoa/mL, >80% motile and <10% abnormal spermatozoa were used. The selected samples were pooled to eliminate individual differences. Different concentrations of ginger extract consisting of 20, 40, 60, and 80 µg/mL were added to semen samples at 4°C. Motility, membrane integrity, and viability were determined at 0, 24, 48, and 72 hours after storage, and DNA fragmentation was solely calculated at 48 h after storage.

Sperm viability and abnormality

The sperm viability and abnormality were determined through eosin-nigrosin staining. Similar proportions of diluted semen and eosin-nigrosin dye (10 µL) were mixed on a warm slide, and a smear of this mixture was prepared on the same slide. The stained seminal smears were observed under a light microscope with 40 X (Nikon, Japan). The sperm with detached heads, abaxial heads, malformed heads, bent tails, coiled tails, double tails, and protoplasmic droplets were considered as abnormal sperm and unstained sperm cells were considered alive (Hosseini *et al*., 2016).

Plasma membrane integrity

To evaluate sperm plasma membrane integrity, hypo-osmotic swelling (HOS) test was used according to the method of (Santiago-Moreno *et al*., 2009)). In brief, a drop of semen sample (5 µL) was added to 200 µL of 100 mOsm/kg of HOS solution (The mixture of fructose [9 g/L] and sodium citrate [4.9 g/L] in distilled water) and incubated at 37°C for 30 min. Afterward, a portion of the semen sample was placed on a slide, covered with a coverslip. The percentage of 200 swollen tail sperms was determined by a light microscope (Nikon, Japan).

DNA fragmentation test

Microtubes containing agarose were melted at 100°C for 5 min. An aliquot of 100 µL of diluted semen sample was mixed at 37°C with melted agarose. The mixture was pipetted onto glass slides pre-coated with melting agarose, covered with coverslips, and kept on an ice pack for 10 min to solidify. Immediately coverslip removed. The slides were immersed horizontally in a tray containing acid denaturation solution in the dark condition then were neutralized at room temperature. The slides washed, dehydrated in sequential 70%, 90%, and 100% ethanol, air-dried, and stained. The stained slides were evaluated by counting 200 sperm on each slide for halo size and dispersion pattern. The nuclei with large to medium size halo belonged to sperms with non-fragmented DNA, while nuclei with small size halo or without a halo belonged to sperms with fragmented DNA according to Hooshmand fan avar kit manual, Iran (Figure 1). Moreover, a fragmentation rate of < 25% in each sample was considered normal (Fernandez *et al*., 2005).

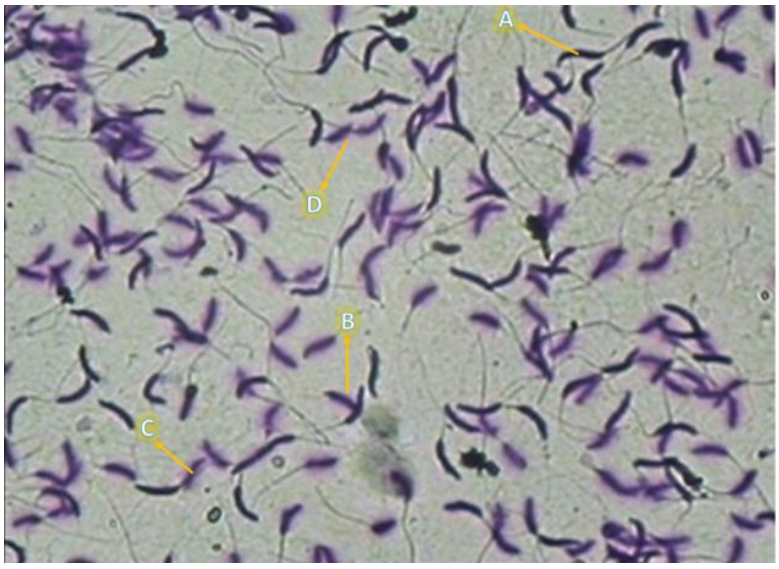

Figure 1. Sperms with different halo sizes: A-No halo; B-Small halo; C-Medium halo; D-Large halo.

Statistical Analysis

Data of the experiment were analyzed by GLM procedure of SAS software and means of treatments were compared with Duncan multiple range tests ($P < 0.05$).

Results

Sperm viability

Sperm viability percentage is depicted in Table 1. A dramatic decreasing trend was noted between treatments. The recorded sperm viability varied from 36% (80 µg/mL) to 70.50% (20 µg/mL) at 48 h after semen collection. The ginger extract was improved sperm viability percentage significantly. After 72 hours, no significant differences were observed between treatments.

Table 1. The effect of various concentrations of ginger extract on rooster sperm viability during liquid storage at 4°C

Ginger extract (µg/mL)	Time (hours)			
	0	24	48	72
0 (Control)	$82.33^{a} \pm 3.79$	$80.38^{a} \pm 1.51$	$76.00^{a} \pm 2.28$	25.50 ± 2.88
20	$83.33^{a} \pm 2.01$	$87.00^{a} \pm 3.46$	$70.05^{ab} \pm 3.88$	28.61 ± 2.05
40	$79.66^{a} \pm 1.05$	$67.05^{ab} \pm 5.59$	$55.67^{b} \pm 6.52$	20.38 ± 3.40
60	$82.83^{a} \pm 1.51$	$54.38^{b} \pm 7.86$	$37.33^{c} \pm 5.40$	22.05 ± 4.25
80	$74.16^{b} \pm 8.36$	$40.00^{c} \pm 4.75$	$36.00^{c} \pm 4.09$	31.00 ± 4.00

Means ± SD; ^{abc} Means within the same column with different superscript letters differ significantly ($P < 0.05$).

Sperm motility

After 48 hours, a 13% improvement in sperm motility was noted in samples added 20 µg/mL of ginger extract when compared to the control group (Table 2).

Table 2. The effect of various concentrations of ginger extract on rooster sperm motility during liquid storage at 4°C

Ginger extract (µg/mL)	Time (hours)			
	0	24	48	72
0 (Control)	82.33 ± 3.79	$58.20^{b} \pm 2.42$	$63.61^{b} \pm 1.47$	$25.83^{b} \pm 2.82$
20	83.61 ± 1.24	$74.61^{a} \pm 5.75$	$76.05^{a} \pm 3.09$	$28.61^{b} \pm 2.05$
40	83.05 ± 9.50	$69.66^{a} \pm 3.73$	$65.16^{b} \pm 2.32$	$14.38^{b} \pm 1.32$
60	80.33 ± 2.02	$71.33^{a} \pm 2.49$	$35.61^{c} \pm 1.92$	$46.66^{a} \pm 6.68$
80	87.00 ± 1.41	$70.00^{ab} \pm 4.91$	$24.05^{d} \pm 2.89$	$19.33b \pm 3.72$

Means ± SD; ^{abc} Means within the same column with different superscript letters differ significantly ($P < 0.05$).

Membrane integrity

Membrane integrity was significantly affected by adding ginger extract (Table 3). The highest and lowest value was observed 58.66% (60 µg/mL) and 30.5% (control) at 48h, respectively. There was no significant difference between the four treatments and control at 72 h.

Table 3. The effect of various concentrations of ginger extract on rooster sperm membrane integrity during liquid storage at 4°C

Ginger extract (µg/mL)	Time (hours)			
	0	24	48	72
0 (Control)	86.61^{a} ± 1.32	81.66^{a} ± 1.42	30.05^{c} ± 1.96	40.00 ± 2.62
20	75.38^{a} ± 3.74	57.38^{b} ± 8.34	34.66^{bc} ± 4.85	33.05 ± 6.66
40	28.00^{b} ± 2.19	60.38^{b} ± 6.70	47.66^{ab} ± 5.03	37.61 ± 3.40
60	38.00^{b} ± 1.26	65.38^{ab} ± 6.94	58.66^{a} ± 3.75	30.33 ± 4.13
80	81.61^{a} ± 3.46	58.33^{b} ± 6.08	51.66^{a} ± 9.27	43.38 ± 6.27

Means ± SD; ^{abc} Means within the same column with different superscript letters differ significantly ($P < 0.05$).

Sperm DNA fragmentation (SDF)

The SDF was significantly lower when the ginger extract was supplemented at 20 and 40 µg/mL compared with others (Figure 2).

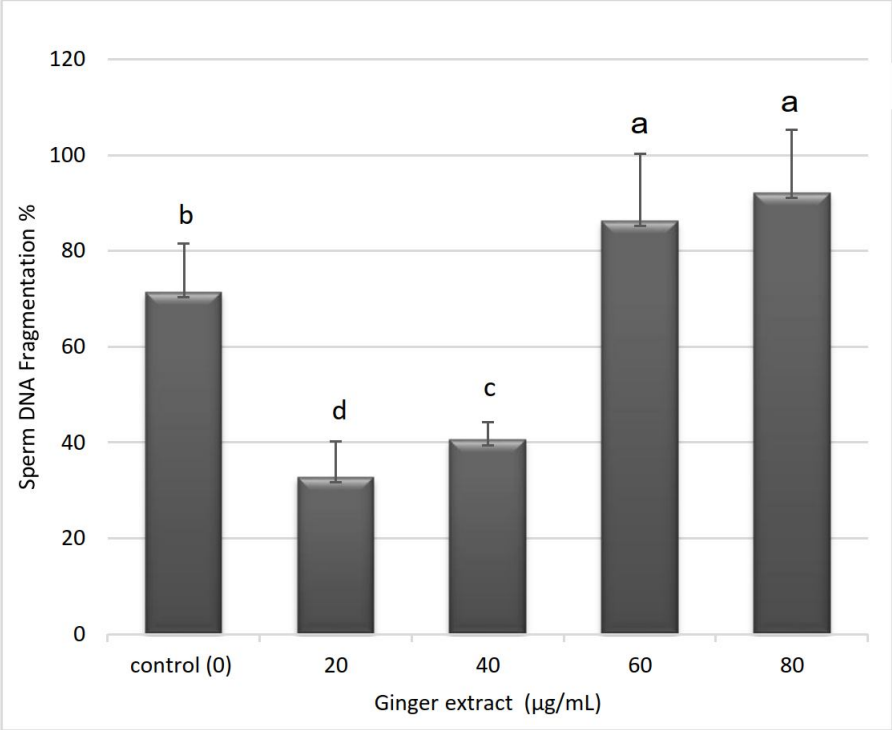

Figure 2. Sperm DNA fragmentation, different concentrations of ginger extract in semen roosters. Means ± SD.

Discussion

Environmental factors such as stress, disease, and malnutrition can lead sperm towards apoptosis and thereby sperm death. The reduction of molecular oxygen (O_2) produces superoxide, which is the precursor of different types of reactive oxygen species. The unpaired electrons, such as superoxide radical ($O^{\circ}{}_2^{-}$), Nitrite Oxide, and hydroxyl radical ($^{\cdot}OH$) can cause significant damage to cell structures (Palacio and Mooradian, 2016). The poultry sperm has a high proportion of unsaturated fatty acids that makes it more susceptible to lipid peroxidation. Ginger contains 6-gingerol 6-paradol, phenolic1, 3-diketones, and zingerone, which have a protective effect against lipid peroxidation (Hegazy *et al*., 2016). The first major finding of the current experiment was establishing a negative correlation between the application of ginger and sperm DNA fragmentation. The study shows that ginger extract 20 µg/mL caused a significant reduction in DNA fragmentation. This is the first study reporting the protective effect of ginger extract upon sperm DNA in rooster. The beneficial effect of ginger on DNA fragmentation was also reported in the other study in which oral administration of ginger powder significantly decreased lipid peroxidation, while amplified the serum glutathione and the quantity and quality of human sperm (Hosseini *et al*., 2016).

The addition of ginger extract at 20 µg /mL improved sperm motility and viability after 48 hours of storage at 4°C. Our findings correspond with results reported for the effect of silymarin on rooster semen at 4°C (Ziaeirad *et al*., 2016). ROS and lipid peroxidation can impair the mitochondrial membrane,

decrease ATP levels, and damage sperm axoneme, which ultimately reduces sperm motility (Sanocka and Kurpisz, 2004). It seems that the antioxidant property of ginger extract reduces the toxic effects of ROS in semen and improves sperm characteristics during storage at 4°C. The effect of ginger and cinnamon combinations on infertile diabetic rats, increased sperm motility, viability, and reproductive behavior (Hafez, 2010). Antioxidant compounds of ginger extract improved all reproductive factors of epileptic mice by Lamotrigine (Poorrostami and Farokhi, 2016). Oral administration of Ginger rhizome powder also enhanced sperm motility in rats (Khaki *et al.*, 2009)Flavonoid compounds inhibit oxidation and disintegration of the cell membrane by affecting the polar groups of membrane phospholipids. The optimum performance of antioxidants occurs at certain concentrations, with increasing concentration, its function gets reversed and induces oxidation (Cao and Cutler, 1993). Although the reason has not been cleared yet the intake of antioxidant supplements was recommended in the condition of deficiency (Salehi *et al.*, 2018). The results of this experiment showed that the use of ginger extract at concentrations higher than 40 µg/mL had adverse effects on viability, motility, and membrane integrity.

Antioxidants break down oxidative reactions and thereby reduce oxidative stress (Bansal and Bilaspuri, 2011). In infertile individuals, sperm DNA fragmentation and ROS were increased (Fanaei *et al.*, 2011). Indeed, ginger was found to increase the activity of the antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) in different male reproductive tissues/organs, mainly epididymis, testis, and prostate (Banihani, 2019).

Sperm is highly susceptible to oxidative attack because it lacks significant antioxidant protection due to the limited volume and restricted distribution of cytoplasmic space. In particular, sperm membrane lipids are susceptible to oxidative stress because they abound in significant amounts of polyunsaturated fatty acids. The results of the study indicated that the addition of alcoholic extract of ginger in a definite amount can improve sperm motility and lead to reduced sperm DNA fragmentation.

Acknowledgments

This work was supported by Grant-in-Aid for Scientific Research from Lorestan University.

ORCID

Maryam Sharafi
https://orcid.org/0000-0001-9105-3447

Saied Mohammadzadeh
https://orcid.org/0000-0002-7791-9272

Seyyed Mojtaba Mousavi
https://orcid.org/0000-0003-2771-3150

Hasan Mumivand
https://orcid.org/0000-0003-2126-8092

References

Akhlaghi A, Ahangari YJ, Navidshad B, Pirsaraei ZA, Zhandi M, Deldar H, Rezvani MR, Dadpasand M, Hashemi, SR, Poureslami R & Peebles ED. 2014. Improvements in semen quality, sperm fatty acids, and reproductive performance in aged Cobb 500 breeder roosters fed diets containing dried ginger rhizomes (*Zingiber officinale*). Poultry Science, 93:1236-1244. DOI: 10.3382/ps.2013-03617

Altman RD & Marcussen KC. 2001. Effects of a ginger extract on knee pain in patients with osteoarthritis. Arthritis & Rheumatism, 44: 2531-2538. DOI: 10.1002/1529-0131

Banihani SA. 2019. Effect of ginger (*Zingiber officinale*) on semen quality. Andrologia, 51: e13296. DOI: 10.1111/and.13296

Banks S, King SA, Irvine DS & Saunders PT. 2005. Impact of a mild scrotal heat stress on DNA integrity in murine spermatozoa. Reproduction, 129: 505-514. DOI: 10.1530/rep.1.00531

Bansal AK & Bilaspuri GS. 2011. Impacts of oxidative stress and antioxidants on semen functions. Veterinary Medicine International. DOI: 10.4061/2011/686137

Bode AM & Dong Z. 2011. The Amazing and Mighty Ginger. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; Chapter 7.

Cao G & Cutler RG. 1993. High concentrations of antioxidants may not improve defense against oxidative stress. Archives of Gerontology and Geriatrics, 17: 189-201. DOI: 10.1016/0167-4943

Fanaei H, Keshtgar S, Bahmanpour S, Ghannadi A & Kazeroni M. 2011. Beneficial effects of alpha-tocopherol against intracellular calcium overload in human sperm. Reproduction Science, 18: 978-982. DOI: 10.1177/1933719111401656

Fernandez JL, Muriel L, Goyanes V, Segrelles E, Gosalvez J, Enciso M, LaFromboise M & Jonge CD. 2005. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertility and Sterility Journal, 84: 833-842. DOI: 10.1016/ j. fertnstert. 2004.11.089

Garcia-Salas P, Morales-Soto A, Segura-Carretero A & Fernandez-Gutierrez A. 2010. Phenolic-compound-extraction systems for fruit and

vegetable samples. Molecules, 15: 8813-8826. DOI: 10.3390/molecules15128813

Hafez DA. 2010. Effect of Extracts of Ginger Goots and Cinnamon Bark on Fertility of Male Diabetic Rats. Journal of American Science, 6: 940-947.

Hegazy AM, Mosaed MM, Elshafey SH & Bayomy NA. 2016. 6-gingerol ameliorates gentamicin induced renal cortex oxidative stress and apoptosis in adult male albino rats. Tissue Cell, 48: 208-216. DOI: 10.1016/j.tice.2016.03.006

Hosseini JA, Mardi Mamaghani H, Hosseinifar MA, Sadighi Gilani F, Dadkhah D & Sepidarkish M. 2016. The influence of ginger (*Zingiber officinale*) on human sperm quality and DNA fragmentation: A double-blind randomized clinical trial. International Journal of Reproduction Biomedicine, 14: 533-540.

Khaki A, Fathiazad F, Nouri M, Afshin Khaki A, Ozanci CC, Ghafari-Novin M & Hamadeh M. 2009. The effects of Ginger on spermatogenesis and sperm parameters of rat. International Journal of Reproductive Bio Medicine, 7: 7-12.

Meutia Z. 2018. Identification of Zingiberaceae as medicinal plants in Gunung Cut Village. Aceh Barat Daya, Indonesia Journal of Tropical Horticulture, 1: 24-28. DOI: 10.33089/ jthort. v1i1.9

Palacio C & Mooradian A. 2016. Clinical trials and antioxidant outcomes. Oxidative Stress and Antioxidant Protection. The Science of Free Radical Biology and Disease, 493-506. DOI: 10.1002/9781118832431.ch30

Poorrostami A & Farokhi F. 2016. The Effect of Hydroalcoholic Extract of Ginger on Reproductive System of Epileptic Female Rats Treated with Lamotrigine. Qom University Medicine Science Journal, 10: 36-47.

Salehi B, Martorell M, Arbiser JL, Sureda A, Martins N, Maurya PK, Sharifi-Rad M, Kumar P & Sharifi-Rad J. 2018. Antioxidants: Positive or Negative Actors? Biomolecules, 8: 124. DOI: 10.3390/biom8040124

Sanocka D & Kurpisz M. 2004. Reactive oxygen species and sperm cells. Reproduction Biology and Endocrinology, 2:12. DOI:10.1186/1477-7827-2-12

Santiago-Moreno J, Castaño C, Coloma MA, Gómez-Brunet A, Toledano-Díaz A, López-Sebastián A & Campo JL. 2009. Use of the hypo-osmotic swelling test and aniline blue staining to improve the evaluation of seasonal sperm variation in native Spanish free-range poultry. Poultry Science, 88: 2661-2669. DOI: 10.3382/ps.2008-00542

Slameňová D, Kubošková K, Horváthová E & Robichová S. 2002. Rosemary-stimulated reduction of DNA strand breaks and FPG-sensitive sites in mammalian cells treated with H2O2 or visible light-excited Methylene Blue. Cancer Letters, 177: 145-153. DOI: 10.1016/ S0304-3835(01)00784-4

Surai PF, Blesbois E, Grasseau I, Chalah T, Brillard JP, Wishart GJ, Cerolini S & Sparks NHC. 1998. Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 120: 527-533. DOI: 10.1016/s0305-0491(98)10039-1

Yanishlieva NV and Marinova EM, 1996. Antioxidative effectiveness of some natural antioxidants in sunflower oil. Zeitschrift für Lebensmittel-Untersuchung und Forschung, 203: 220–223. DOI: 10.1007/BF01192867

Ziaeirad H, Roostaei M & Mohammadi M. 2016. Effect of silymarin on rooster semen during storage at 4 °C. Journal of Animal Science Researches, 26: 1-13.