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Original Article

The Effect of Oral Administration of Zinc Oxide Nanoparticles on Quantitative and Qualitative Properties of Arabic Ram Sperm and Some Antioxidant Parameters of Seminal Plasma in the Non-Breeding Season

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ABSTRACT

Zinc is an essential mineral and accepted as a trace element in the animal nutrition and for its role in biological enzymatic pathways. This study aimed to investigate the effect of oral administration of zinc oxide nanoparticles (ZnONPs) on some of the antioxidant parameters of semen plasma, quantitative and qualitative properties of Arabic ram sperm in the non-breeding season. Twelve adult Arabic rams (about 3-5 years old, 70 ±2.1 kg) were randomly assigned to receive one of the three levels of dietary ZnONPs (control; 0, group 1; 40 ppm and group 2; 80 ppm). Results showed that using different levels of ZnONPs increased the activity of superoxide dismutase enzyme (SOD) and total antioxidant capacity (TAC) of semen plasma significantly compared with the control group ($P<0.05$). Motility (74.83%), viability (76.90%), semen volume (1.76 ml) and sperm concentration (1418×10^6/ml) were significantly ($P<0.05$) higher in ZnONPs supplemented groups compared with the control group. The sperm morphological abnormalities reduced significantly in treated groups (10.46 and 9.07%) compared with the control group (12.66%; $P<0.05$). Also, the results suggested that 80 ppm level of ZnONPs increased the functionality of sperm membrane (44.38%) compared with other groups (37 and 35.66%, respectively for groups 1 and control) ($P<0.05$). Based on the results, using 80 ppm level of ZnONPs lead to an improvement in the activity of superoxide dismutase enzyme (48.62 ml) and total antioxidant capacity of semen plasma (111.88 µg/ml) compared with other groups ($P<0.05$). In conclusion using 80 ppm level of ZnONPs had a positive effect on the quantitative and qualitative properties of sperm and lead to a significant betterment in the activity of some antioxidant parameters of Arabic ram semen in the non-breeding season.

Keywords: Ram, Sperm, Superoxide dismutase, Total antioxidant capacity, Zinc oxide nanoparticles

L'effet de administration orale des nanoparticules d'oxyde de zinc sur les propriétés quantitatives et qualitatives du sperme ainsi que sur les paramètres antioxydants du liquide séminal chez le mouton arabe en-dehors de sa saison de reproduction

Résumé: Le zinc est un minéral essentiel étant considéré comme un oligo-élément dans la nutrition animale, étant donné son rôle dans les voies biologiques enzymatiques. L'objectif de cette étude était d'étudier l'effet de l'administration orale des naoparticules d'oxyde de zinc (ZnONPs) sur les paramètres antioxydants du

liquide séminal, ainsi que sur les propriétés quantitative et qualitative du sperme du mouton arabe en-dehors de la saison de reproduction. Un total de 12 moutons arabes adultes a été sélectionné de façon aléatoire pour recevoir l'une des 3 concentrations de ZnONPs (control; 0, groupe 1; 40 ppm and groupe 2; 80 ppm). Nos résultats ont démontré l'effet stimulant de l'administration des différents taux de zinc sur l'activité de l'enzyme superoxide dismutase (SOD) ainsi que sur la capacité antioxydante totale (TAC) du liquide séminal, ces paramétres montrant une augmentation significative comparé au groupe control (P<0.05). La motilité (74.83%), la viabilité (76.90%), le volume du liquide séminal (1.76 ml) et la concentration de sperme (1418×106/ml) étaient significativement plus élevés dans les groupes traités avec ZnONPs comparés au groupe control (P<0.05). De plus, les malformations observées au niveau des spermes des groupes traités (10.46 and 9.07%) étaient mois importantes par rapport au groupe control (12.66%; P <0.05). Nos résultats ont également révélé qu'une supplémentation de 80 ppm de ZnONPs augmente de façon significative la fonctionnalité de la membrane des spermes (44.38%) comparés aux groupes 1 (37) et control (35.66%) (P<0.05). De plus, selon nos résultats, une supplémentation en ZnONPs à une concentration de 80 ppm contribue à l'amélioration de l'activité enzymatique de la superoxide dismutase (48.62 ml) ainsi que de la capacité antioxydante totale du liquide séminal comparé (111.88 µg/ml) au groupe control (P<0.05). En conclusion, une supplémentation de 80 ppm de ZnONPs montre un effet positif sur les propriétés quantitatives et qualitatives du sperme et peut mener à une amélioration significative des paramètres antioxydants du liquide séminal chez le mouton arabe en-dehors de sa saison de reproduction.

Mots-clés: Mouton, Sperme, Superoxyde dismutase, Capacité antioxydant totale, Nanoparticules d'oxyde de Zinc

INTRODUCTION

The sperm membrane is prone to lipid peroxidation because of containinga high amount of unsaturated fatty acids (Alvarez et al., 1987). In this regard, the ram sperm is more susceptible to the oxidative stress than other species (Baumber et al., 2000). Zinc is a major element in the whole body cells and involves a wide range of metabolic processes. This element plays a vital role in many activities of enzymatic systems, and is well known for sperm production and maturation. As a scarce element, zinc is directly related to semen parameters (Chvapil, 1973). Semen contains a high concentration of zinc which plays an essential role in the removal of anion superoxide produced by malformed ejaculated spermatozoa. Moreover, this element is needed for better function of reproductive system in the male (Zhang et al., 2017). Prasad et al. (2004) reported that high amount of zinc in mammals' semen is of critical importance for Spermatogenesis. Various methods have recently applied for ZnONPs production. These methods include micro-emulsion, colloidal synthesis, sedimentation, sol-gel method and thermal spraying (Kołodziejczak-Radzimska and Jesionowski, 2014; Al-Mubaddel et al., 2017). ZnONPs are applied in food industry, pharmacy, rubber productions, electronic industry and food additives because of its unique properties (Song et al., 2010). Diminishing nano-particles and increasing surface-to-volume ratio in nano-compound increase their contact with other molecules, thus it leads to different chemical reactions of organic and non-organic molecules. These chemical reactions are unknown in many cases. In some studies about diabetic rats, ZnONPs causes an increase in anti-oxidant enzymes and also improvement of qualitative parameters of sperm (Afifi et al., 2015; Nazarizadeh and Asri-Rezaie, 2016). These studies suggested that ZnONPs have considerable antioxidant properties. There have been various studies about the role of zinc on reproductive system of farm animals (Rahman et al., 2014), but there have not been reported about its role on the reproductive functions and antioxidant activity in farm animals specially in rams with the levels considered in this study. Most studies suggested that this element was used in vitro as an additive to the sperm extenders (Barkhordari et al., 2013; Song et al., 2014). As the nano compounds are used increasingly in different industries and the

uncertainties about the effect of this mineral on different organs of livestock, it seems necessary to evaluate the possible effect of ZnONPs as an oral supplement in livestock rations.With regard to the above mentioned contents, this study aimed to investigate the effect of oral administration of ZnONPs on the quantitative and qualitative properties of sperm and some antioxidant parameters of Arabic ram seminal plasma in the non-breeding season. In countries like Iran (located in an average latitude), seasonal changes are the factors restricting sheep reproductive functions. Although the reproductive system of rams are affected by the seasonal changes less than ewes, some studies reported the effects of these changes on testicular volume, hormonal secretion, sexual behaviors and semen quality (Casao et al., 2010). Thus, in the current study the possible positive effects of oral administration of ZnONPs on rams reproductive performance and the potential alleviation of divers' effects of non-breeding season on Arbic ram semen quality were investigated.

MATERIALS AND METHODS

Feeding and caring for the livestock. The present study has been done in Ramin Animal husbandry Station of Agricultural and Natural Resources University located in Mollasani, 36 km far from the north east of Ahvaz, Khoozestan province from the beginning of January to the end of March, 2016. For this purpose, 12 adult Arabic rams (about 3-5 year old, 70 ±2.1 kg) were randomly assigned to receive one of the three levels of dietary ZnONPs (control; 0, group 1; 40 and group 2; 80 ppm). Rams fed with a ration containing barley (64%), alfalfa (10%) and wheat straw (26%) for 49 days (Table 1). For feeding, first the determined levels of ZnONPs were weighted by a digital scale and then embedded in empty capsules. Empty capsules containing ZnONPs were fed to the treatment groups (1 and 2) using medicine feeding gun. The basic ration contained 18.68 ppm zinc. It is needed to mention that ZnONPs was white powder, with 99% purity. The size of particles was 10-30 nm provided by Pishgamane Nano Mavad (Nano material) Iranian, Mashhad Iran.

Sample preparation. Sperm collection was done weekly for a period of 4 weeks through electric stimulation and using electro-ejaculator machine 42 days after food consumption (spermatogenesis takes 42-49 days in rams). The ejaculated semen was collected in graded-isothermal falcons (37 °C), and the semen volume was recorded. The samples of each livestock were transferred immediately to the lab and analyzed separately. Finally, the leftovers of each sample were centrifuged at 4000 rpm for 5 min. The semen samples kept at -20 °C until the time of measuring the enzymatic activity of superoxide dismutase and total antioxidant capacity.

Sperm Evaluation. To evaluate the qualitative and quantitative properties of sperm, immediately after sampling, the semen of each livestock was diluted using normal Saline (0.9 Sodium Chloride) in a ratio of 1:100.

Measurement of the percentage of progressive motility of sperms. To measure the percentage of sperm motility, a sample of diluted semen was placed on the slide at 37 °C by Pipetta Pasteur. Then a cover slip was fixed on it, the percentage of sperms with progressive motility in some microscopic fields was measured by an optical microscope (with ×400 magnification), and the mean of microscopic fields was registered as the percentage of progressive motilityof sperms on that slide (Kreider et al., 1985).

Evaluation of the percentage of live and dead sperms and morphological abnormalities of sperms. Eosin-Nigrozine was used to evaluate the percentage of live/dead sperms and the abnormal sperms. Ten grams of Nigrozine dissolved in 10 ml boiling distilled water, then passed through the filter paper for preparing the staining solution. Therafter, 0.7 g Eosin was added to that solution. Finally, 0.9 g sodium chloride was used for fixing the osmotic pressure of the cell in staining solution, and the ultimate volume became 100 ml. The

prepared stain was kept at 5 °C. The semen of each treatment group mixed with Eosin-Nigrozine stain (1:1) and placed on a slide after 30 seconds and dried by hot air flow. After preparing and drying smear, the slides were measured by an optical microscope (with ×1000 magnification) and immersion oil. The percentage of live and dead sperms was calculated in some slides (by counting at least 200 sperms in each slide). The dead sperms absorbed Eosin and went pink; while the live sperms did not absorb the stain and remained white. To measure the percentage of abnormal sperms, the physical damages to the healthy sperms were tried to be avoided while preparing smear, otherwise the number of abnormal sperms would increase. The percentage of abnormal sperms was analyzed to measure the percentage of live and dead sperms (Kreider et al., 1985).

Evaluation of membrane integrity. The integrity of sperm plasma membrane was evaluated by HOST assay. The hypoosmotic solution was prepared by dissolving 0.735 g of Tri-sodium citrate dehydrate and 1.351 g of fructose in 100 ml of double-distilled water. First, 500 µl of hypoosmotic solution was mixed with 50 µl of each specimen and incubated at 37 °C for 45 minutes, then the smear was prepared by picking up one drop of the premixed sample and monitored by optical microscope after drying samples. In each slide, 200 sperms were counted and the percentage of sperm in shape of the twisted tail and swollen head which shows the normality of sperm membrane was measured (Adeel et al., 2009).

Quantification of the sperm concentration and pH of semen. Hemocytometer slide (×400 magnification) was used to measure the concentration of sperm. For this purpose, the semen samples were diluted in physiologic serum in a ratio of 1:100 and a drop of formalin was added to each test tube to make the spermatozoa immotile on the slides. A clean hemocytometer slide was placed under the microscope and a clean cover slip was laid on it, the gap between the slide and the cover slip was 0.1 mm^3. Then, one drop of diluted semen was placed in the corner of the slide with a pipett pasteur. The sample spread out on the entire surface of the slide. After waiting for 5 minutes, sperms became utterly motionless on the slide, and finally they were counted. There is a square in the center which is divided into 25 big squares. Each big square is divided into 16 small squares. For the convenience and accurate measurement, five big squares were counted, and then the concentration of sperm was calculated. Semen pH was measured before adding the dilutor and immediately after sampling.

Measurement of the activity of Superoxide dismutase. The activity of Superoxide dismutase enzyme was measured by Randox kit (England, Manchester), based on McCord and Fridovich (1969). This enzyme neutralizes the toxic superoxide radicals - generated during energizing reactions-makes oxidative and converts them into hydrogen peroxide and molecular oxygen. First, to measure the enzyme activity, some Xanthine oxidase (with the absence of homogenate containing SOD) was added into Cuvettes containing the Reaction mixture (0.005 mM xanthine, 0.001 Mm ferricytochrom). Then, the enzymatic activity was measured at a wavelength of 550 nm and absorption length of 0-0.1 to get enough Xanthine oxidase for creating ΔOD=25 mABs/min. Xanthine oxidase solution must be sufficient so that anion superoxide produced by Xanthine can increase the reduction capacity of Cyt c (III) to ΔOD level. In this method, Xanthine and Xanthine oxidase were used for producing superoxide radicals. These produced radicals reacted with INT which was found in the kit and made Redformazen dye. Then, the activity of Superoxide dismutase enzyme was evaluated by measuring the percentage of inhibition of this reaction. One unit of SOD is the amount of enzyme which can inhibit 50 percent of restoration pace of INT in test conditions.

Measurement of the total antioxidant capacity (TAC) of semen. FRAP measures total antioxidant capacity (TAC) of semen. This method is based on the capability of semen antioxidants to convert Fe^{2+} to Fe^{+} which suggests the total antioxidant activity. The results reported as micro molar of Fe^{2+} per liter of

semen. For this purpose, FRAP was made as follows: 10 ml acetate buffer and 1 ml Tripyridyltriazine (TPTZ) were mixed with HCl. Then, 1 ml Ferric chloride was added to the mentioned solution. Thereafter , 1.5 ml FRAP was added to each well and incubated in water bath at 37°C for 5 min, then 50 µl of the standard solution with specified concentrations plus 50 µl of distilled water was added to the wells and re-heated in a water bath for 10 minutes. After picking up the test kit from the water bath and formulating the colorful complex, Spectrophotometer showed the wavelength of 593 Nm, and FRAP was converted to µmol Trolox equivalent/L by Trolox standard and expressed based on µmol/ml.

Statistical Analysis. Data obtained from this study were analyzed randomly through variance (SPSS20) and the means of dietary ZnONPs (control, 0; group 1, 40; group 2, 80 ppm) were compared using Duncan's test in probability level of 5%.

Table 1. The chemical composition of the ration

Ingredients	Alfalfa (10%)	Barleycorn (64%)	Wheat straw (26%)
DM%	92	89	91
Zn (ppm)	24	23	6
Ca (DM%)	1.4	0.1	0.039
P (DM%)	0.2	0.4	0.08
CP (%DM)	17	12	3.6
Metabolism energy (Mcal/Kg)	3	2	1.5

RESULTS

Table 2 shows the results from the evaluation of the effect of ZnONPs on the quantitative and qualitative properties of Arabic ram sperm. The motility, viability, the sperm concentration and the semen volume in two levels of dietary ZnONPs (group 1, 40 ppm; group 2, 80 ppm) increased compared with the control group ($P<0.05$). The control group had the highest level of sperm morphological abnormalities ($P<0.05$). The percentage of normality of the sperm plasma membrane in two levels of dietary ZnONPs (40 ppm and 80 ppm) was more than that of the control group ($P < 0.05$). The effect of different levels of ZnONPs on the activities of Superoxide dismutase enzyme and total antioxidant capacity of semen plasma is shown in Table 3. The level of 80 ppm ZnONPs increased the activity of Superoxide dismutase enzyme and total antioxidant capacity of semen plasma increased in group 2 compared to control and group 1 ($P<0.05$). However, the results showed that there is not a statistically significant difference between control group and group 1 regarding the mentioned parameters ($P < 0.05$).

DISCUSSION

Zinc plays an essential role in sperm production, viability, prevention of damages to sperm and sperm membrane stability (Lewis-Jones et al., 1996). Studies showed that zinc plays a significant role in stabilization of chromatin structure of sperm nuclei (Gromadzka-Ostrowska et al., 2012). In the present study, supplementing the ration of Arabic rams with ZnONPs did not have a significant effect on the semen pH because of the duration of ZnONPs administration. With regard to short time feeding (49 days), the semen pH was not affected by treated groups. As the semen pH is kept in a normal range and on the other hand, pH originates from secretions of the prostate gland, ZnONPs prevents acid secretion increment from of the prostate gland (Kumar et al., 2006). In the present study, dietary ZnONPs increased the percentage of sperm motility in treated groups rather than the control group. Reactive oxygen species (ROSs) have a deleterious effect on the activities of enzymatic pathways in oxidative-phosphorylation and glycolysis processes or other ATP producing pathways for sperm

Table 2. Effect of Different Levels of ZnONPs (ppm) on Quantitative and Qualitative Parameters of Arabic Rams Sperm in Non-breeding Season

Experimental Group	Motility (%)	Viability (%)	Abnormality (%)	Membrane integrity	pH	Concentration (10^6/mL)	Volume (mL)
Control	61.86±1.28 [c]	62.00±2.40 [c]	12.66±0.67 [b]	35.66±1.04 [b]	6.27±0.1	1232±12.81 [b]	1.03±0.04 [c]
Group 1 (40 ppm)	67.33±1.26 [b]	70.60±1.38 [b]	10.46±0.43 [a]	37.00±1.33 [b]	6.30±0.05	1418±23.18 [a]	1.46±0.05 [b]
Group 2 (80 ppm)	74.83±1.48 [a]	76.90±1.50 [a]	9.07±0.55 [a]	44.38±1.91 [a]	6.33±0.03	1379±22.64 [a]	1.76±0.06 [a]

In each column, Means with different letters have a statistically significant difference (P <0.05).

cells. Therefore, using antioxidant elements like zinc can neutralize the effect of ROSs which consequently improves the efficiency of ATP producing pathways (Baumber et al., 2000). With regard to the antioxidant activity of zinc and its role in the stabilization of sperm membrane, it can be concluded that this element can play a critical role in viability and motility of sperm. Based on the reposrts, improvement in the motility of sperm results from the increment of the activity of some enzymes like lactate dehydrogenase and sorbitol dehydrogenase, since zinc served as a co-factor for the activities of these enzymes (Rahman et al., 2014).

Table 3. Effect of different levels of ZnONPs (ppm) on superoxide dismutase enzyme activity (SOD) and total antioxidant capacity of Arabic ram semen in non-breeding season

Experimental Groups	SOD (U/mL)	Total antioxidant capacity (µmol/lit)
Control	32.98±1.67 [b]	91.36±7.99 [b]
Group 1(40 ppm)	36.35±0.97 [b]	93.43±4.03 [b]
Group 2(80 ppm)	48.62±1.09 [a]	111.88±1.38 [a]

In each column, Means with different letters have a statistically significant difference (P <0.05).

In this regard, in human in vitro studies pointed out that the ZnONPs has some positive effects on the quality of sperm (Isaac et al., 2017) which is consistent with the results of this study. Although a study indicates some toxic and destructive effects of zinc oxide nanoparticles in vivo and in vitro (Wang et al., 2008), the present study did not reveal the mentioned effects, it may relate to the particle size and the level of administrated ZnONPs. In the present study, the size of ZnONPs was 10-30 nm which may not have any toxic effect on the Spermatogenesis process of Arabic ram. In the current study, ZnONPs increased the sperm viability significantly compared with the control group. One of the reasons of this betterment was the integrity and stability of sperm membrane. Zinc causes the stability and finally the viability of sperm by preventing enzymes, proteins and other vital elements of sperm cells from damages (Hernandez-Sierra et al., 2008). Moreover, zinc stabilizes Ribosomes, lysosomes, DNA and RNA which help the survival and natural function of the sperm cell. Rahman et al. (2014) have done a study on goats. They concluded that administration of 50 and 100 mg zinc (from the source of Zinc sulfate) increases the motility and viability of goat sperm significantly compared with control group which was consistent with the results of this study. However, some studies suggest the adverse effects of ZnONPs on the quality of sperm. Abbasalipourkabir et al. (2015) reported that using over 50 mg/kg of ZnONPs decreases the quality of rat sperm. Their results were inconsistent with those of the present study, this conflict could be related to the difference between animal species in their study and the one in our study, and the manner of drug taking and penetration of nanoparticles into the Blood-testicular barrier (Lan and Yang, 2012). Based on the obtained results of this study, ZnONPs causes the normality of sperm membrane. Zinc replaces Iron III with Iron II and stops Iron from penetrating into ROS production cycle or Fenton reactions, so it protects lipid structure of membrane from free radical attack. Kumar et al. (2006) have done studies on hybrid cows and declared that using zinc sulfate increases the normality of sperm membrane. Sperm membrane has a negative charge,

and ZnONPs have it either, so it cannot penetrate into the sperm membrane and blocks the Lipid peroxidation by creating a protective layer around it (Isaac et al., 2017). The results of this study suggested that ZnONPs significantly reduced the sperm morphological abnormalities in treated groups compared with the control group. There are few studies about the effect of zinc on morphological abnormalities of sperm, Ueda et al. (1991) suggested that abnormal morphology of sperm depends entirely upon the stages of spermatogenesis which is regulated by sertoli cells. As zinc effects on sertoli cells through decreasing and increasing FSH hormone (Gromadzka-Ostrowska et al., 2012), it can consequently be useful to reduce the percentages of loss of morphologicaly abnormal sperms. In the present study, the semen volume in treated groups supplemented with ZnONPs increased more than that in control group. Likewise, researchers reported that using dietary zinc increases the volume of semen in goats (Ibrahim and Yousri, 1992) and rabbits (Moce et al., 2000). The testicular, epididymis, prostate, and the other secondary sexual gonads secretions are responsible to increase the semen volume (Kumar et al., 2006). Zinc is vital for the growth and development of primary and secondary sexual organs, and when there is not zinc in rams' diet, they will face atrophy in these critical organs (Martin et al., 1994). Therefore, ZnONPs consumption in non-breading season provides the optimum condition for these organs to produce more semen in each ejaculate. In the present study, results suggested that ZnONPs have a significant and positive effect on spermatogenesis process which is consistent with those of studies on hybrid cows (Kumar et al., 2006) and men (Wong et al., 2002). Sperm production requires extensive cell division. Zinc plays a vital role in this phenomenon by affecting on meiotic and mitotic cell divisions, DNA synthesis and the activity of DNA / RNA polymerase. Moreover, zinc helps to encode transcription factors related to spermatogenesis (Bedwal and Bahuguna, 1994). This element effects on the activation and maintenance of germinal epithelium in seminiferous tubules and testosterone secretion (Wong et al., 2002). Some recent studies considered ZnONPs as a deletrious factor for rats testicles function (Talebi et al., 2013).To current knowledg there have not been published any study about the effect of ZnONPs on spermatogenesis process in Arabic rams, therefore, the reason for the contradictory reports could be explained as the differential nutritional needs in different animal spicies for spermatogenesis. Thus, further studies in this field are needed. In the present study, it was concluded that 80 ppm level of ZnONPs increased the activity of superoxide dismutase enzyme and total antioxidant capacity (TAC) of semen plasma compared with other groups. Zinc is a scarce and essential element which is needed for activity of more than 200 enzymes and plays a major role in polymer structures such as macromolecules, protein synthesis and cell division (Kumar et al., 2006). Zinc has antioxidant activity and it seems an important factor for anti-bacterial activities in sperm (Gavella and Lipovac, 1998). Zinc of semen plasma stabilizes the membrane and chromatin of sperm and prevents it from breaking down (Chvapil, 1973). It seems that this element acts as a potential scavenger of superoxide anions produced by sperms and white blood cells (Plante et al., 1994). Semen plasma is supposed to have pseudo-antioxidant activity due to the high amount of zinc when it faces so many anion superoxides (Plante et al., 1994). So, this study evaluated total antioxidant capacity (TAC) of semen plasma for more accurate function of zinc in semen plasma, and the results suggested that using 80 ppm level of ZnONPs increased the antioxidant capacity significantly compared with other treated groups. This increase can affect on the qualitative and quantitative parameters of sperm. Hadwan et al. (2012) mentioned that zinc is a significant part of superoxide dismutase enzyme. Studies suggested that zinc acts as a co-factor for many enzymes (Khan, 2011). In a study conducted by Abbasalipourkabir et al. (2015), the results suggested that ZnONPs increased the SOD activity in

rats. Some researchers noted that Zn acts as a powerful antioxidant to decrease the ROS, also zinc supplement reduces lipid peroxidation and finally prevents cells from being destructed (Rogalska et al., 2009). Zinc supllementation may not be related to the activity of superoxide dismutase in a healthy state, but a shortage of dietary zinc could change the activity of this enzyme.

Overall, the results of this study suggested that administration of 80 ppm of ZnONPs in diet of Arabic rams improves the qualitative and quantitative properties of sperm and some antioxidant parameters of seminal plasma in the non-breeding season.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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