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Research Article

The effect of zinc oxide on the glutathione peroxidase and superoxide dismutase enzymes activities of ram sperm at cooling

Hasanain Jihad NEAMAH*[1], Sarah Gatea Fayyadh AL-OMAIRI[2], Mohammed Abdulameer Rashid AL-SARRAY[2]

[1]*Al-Kut Technical Institute, Middle Technical University, Kut, Iraq*
[2]*Department of Animal Production, College of Agriculture - University of Wasit, Wasit, Iraq.*
*Email: hassanain.alkenani@mtu.edu.iq

Abstract

This study was conducted to explore the Zinc oxide on the glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity of the Awassi ram sperm. The semen was divided into four groups, including control by adding Tris extender, T1= Tris extender + 0.01mg Zinc oxide/ml, T2= Tris extender + 0.04 mg\ml Zinc oxide/ml, and T3 = Tris extender + 0.08 mg\ml Zinc oxide/ ml. The diluent semen kept at 5°C. GPx and SOD were measured after reaching to 5°C (Zero time) and 72 hr. The results showed a significant (P<0.05) enhancement of GPx activity at 5°C in the T1 and T2 treatments. There were no significant changes in the T3 and the control group. After 72 hr, the T1 showed the highest GPx activity compared with the control group, whereas, no significant variation between the control and T3 groups. The SOD activity improved significantly (P<0.05) in the T1 and T2 groups as a comparison with the control group. In addition, no significant changes were found among the control and T3 groups at 5 and 72 hr of cooling.

Keywords: Awassi ram, Zinc oxide, GPx, SOD.

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Introduction

The membrane of sperm cells can damage by lipid oxidation because it consists of a high concentration of polyunsaturated fatty acids (PUFA) (Bansal & Bilaspuri 2011; Tran et al. 2017; Al-Sarray et al. 2019). There is a relationship between reactive oxygen species (ROS) formation and cellular oxidative state (Hamilton et al. 2016; Al-Subaihawi et al. 2020; Banana et al. 2021). The low ROS is important for sperm hyperactivity; however, a high level of ROS attacks lipids and changes enzymatic systems (Makker et al. 2009; Al-Sarray et al. 2020), and fails in fertilization (Eidan 2016). Cellular metabolism leads to high ROS accumulation, which leads to DNA damage, membrane fluidity loss, and infertility impairment (Heidari et al. 2019). The cells' anti-oxidant defense system consists of three important enzymes to remove the free radicals, viz. catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (Briggs et al. 2018). Hazarika et al. (2016) referred that adding glutathione to the diluent buck semen protected sperm cells at frozen.

The lipid peroxidation damages the cell membrane and disrupts the ion transfer. In vivo, some studies suggested that zinc acts as a superoxide scavenger. Zinc plays an important function in sperm membrane constancy, sperm motility, tail morphology, and physical properties of the jointed tail's fibers (Heidari et al. 2019). Low zinc concentration increases oxidative damage in the biological environment of semen (Colagar et al. 2009). The most common zinc combination is zinc oxide (ZnO) (Hollis et al. 2005). It acts as an

Table 1. Effect of adding Zinc oxide to the diluent semen on the glutathione peroxidase activity at cooling.

Time	At 5°C	After 72 hr.	*P*-value
Treatments	Mean±SE		
Control	100.33±0.88 cB	64.98±1.21 bA	*
T1	120.02±0.55 aB	70.43 ±0.38 aA	**
T2	116.66±0.88 bB	67.52±0.86 abA	*
T3	101.32±0.66 cB	65.99±1.66 bA	*
P-value	*	**	

The small letters in the columns refer to significant variations among groups. The capital letters in rows refer to significant variations among periods (* = $P<0.05$ and ** = $P<0.01$).

antioxidant by reducing the free radicals (Ozsoy et al. 2011) and MDA level (Dani et al. 2011). Therefore, this study aimed to estimate the effect of zinc oxide on the spermatozoa cells enzymes for Awasi ram's semen at cooling.

Material and Methods

This study was conducted on four Awassi rams (2 years old) with 50±0.16kg. The basal diet was wheat straw + concentration. The ram semen was collected by an artificial vagina (41-42°C) from each ram in the early morning. The training period for rams continued for 5-5 weeks. After collection, the semen was placed in a warm water bath (37°C). Before semen was pooled, a microscopic examination was performed for each sample. The semen was polled to remove individual variations, and then the semen was collected, diluted, and kept at 37°C. The semen was diluted at a rate of 1:10 fresh semen (100µl) and Tris buffer solution (900µl). The Tris buffer solution includes Hydroxymethyl aminomethane, Tris (3.63g), citric acid (1.99g), fructose (0.5g), egg yolk (14.0ml), Penicillin (100000 IU), and Streptomycin (100mg), and distilled water 100m (Evans & Maxwell 1987).

After dilution, the semen was divided into 4 groups, including (control without the addition of zinc + Tris extender, T1 with Tris extender + 0.01mg Zinc oxide/ml, T2 with Tris extender + 0.04mg\ml Zinc oxide/ml, and T3 with Tris extender + 0.08mg\ml Zinc oxide/ml each with three replicates. All tubes were stored at 5°C for 72 hr. The GPx and SOD enzymes were measured after reaching 5°C (at zero time) and after 72 hr. The SOD activity in the spermatozoa was measured according to (Beauchamp & Fridovich 1971).

The data were evaluated using SPSS software. The values are recorded as mean±stander error. The statistical significance between all groups was calculated using one-way ANOVA test. The statistical model design was: $Yij = \mu + Ai + eij$, where Yij= Observation, µ= mean, Ai= the effects of treatments, and eij= Error term.

Results and Discussion

The GPx and SOD are endogenous antioxidants in the seminal plasma (Adewoyin et al. 2017). The results of the current work showed a significant ($P<0.05$) effect on the GPx activity at zero time of 5°C in the T1 and T2 groups, which recorded a lower activity of glutathione, but no significant effect in the control and T3 group at this time. However, post 72 hours of cooling at 5°C, non-significant changes in the control and T3 groups were found, while the T1 group had the highest GPx activity. The significant ($P<0.05$, $P<0.01$) changes were found between groups after 72 hr post-cooling (Table 1).

After reaching 5°C, the SOD activity increased significantly ($P<0.01$) in the T1 and T2 groups, while no significant changes were recorded in the control and T3 groups. After 72 hr, the results showed a significant increase in SOD activity in the T1 group compared with others, while no significant differences were found between T2, T3, and the control group i.e. a significant ($P<0.05$, $P<0.01$) variation were noticed in all groups after 72 hr. post-

Table 2. Effect of adding Zinc oxide to the diluent semen on the super oxide dismutase activity at cooling.

Time	At 5 °C	After 72 hr.	*P*-value
Treatments	Mean±SE		
Control	44.54±0.84 aB	28.51±0.29 abA	*
T1	49.46±0.32 bB	32.00±0.57 cA	*
T2	48.00±0.58 bB	30.56±0.26 bcA	*
T3	45.81±0.69 aB	28.01±1.15 aA	*
P-value	*	*	

The small letters in the columns refer to significant variations among groups. The capital letters in rows refer to significant variations among period
(* = $P<0.05$ and ** = $P<0.01$).

cooling (Table 2). Exogenous antioxidants can enhance spermatozoa in bull (Daghigh-Kia et al. 2014), ram (AL-Sarray 2019), and boar (Paál et al. 2018). Adding antioxidants to the diluent semen improves semen quality at cooling (Zhang et al. 2015). The antioxidants remove radicals and increase spermatozoa viability (Mortazavi et al. 2014).

Our result showed high protection of spermatozoa against oxidative stress during cooling periods using zinc oxide. In addition, a significant activity of GPx of ram spermatozoa after adding zinc oxide is likely associated with the zinc function related to ATP. Zn plays an important role in regulating the energy of its phospholipids in the sperm cells (Ahuja & Parmar 2017). There are high concentrations of polyunsaturated fatty acids (PUFA) in ram's sperm cells, which make the sperm membranes sensitive to damage by free radicals (Neamah & Houbi 2020). Zinc oxide can remove the free radicals caused by different factors, like ionizing radiation, and lipid peroxidation amounts (Dani et al. 2011). Heidari et al. (2019) pointed out that adding 0.1mg/ml of zinc oxide improved the plasma membrane integrity and decreased Malondialdehyde (MDA) in sperm cells and these led to increasing the survival rate during storage. Also, they showed a significant effect of adding zinc oxide to the ram semen on the viability percentage.

Zinc has an important role as a scavenger against free radicals, where it has the ability to inhibit phospholipase and prevent sperm oxidative damage and lipid peroxide formation (Eggert et al. 2002). These properties may account for improved GPx and SOD activity. Bettger & O'Dell (1981) found that zinc stabilizes DNA, lysosomes, and ribosomes which help sperm survival. Zinc also increases lactate dehydrogenase and sorbitol dehydrogenase enzyme activities, and these enzymes have the ability to keep sperm motility (Nagamine et al. 1990). Gabra & Basuini (2018) suggested that zinc oxide supplementation enhanced the reproductive performance of buffalo. The zinc concentration showed a positive correlation with semen quality (Sabhapati et al. 2016). An increase in the SOD and GPx activity and decreased MDA level were shown in zinc oxide-treated rats by Afifi et al. (2018).

This study showed non-significant effects by adding 0.08mg of zinc to extender semen, This result is in agreement with the finding Halo et al. (2021), that Zinc oxide with high concentrations decreased the rabbit sperm motility at cooling. Alavi-Shoushtari et al. (2009) indicated that Zinc concentration imbalance in buffalo sperm or seminal plasma may have a negative effect on oxidative stress increasing and may be responsible for ram infertility. The important effects of the zinc element involve two mechanisms: reduction in the formation of •OH from H_2O_2 and protection of protein sulfhydryls (Powell 2000). The SOD and GPx decreased post 27 hr. of cooling in the current work, this may be caused by the negative correlation between extracellular enzyme activity, and sperm viability, where the activity of the enzymes is impacted by the storage period (Gebreselassie et al. 2013).

As a conclusion, adding 0.04 and 0.01mg/ml of Zinc oxide to dilute ram semen improved significantly superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities at cooling,

and no significant effect was recorded by adding 0.08mg/ml.

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