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Effect of the concentration of vitamin D3 on the Alanine-aminotransferase (ALT) and Aspartate-aminotransferase (AST) enzymes

Hayder Mohammed Hassan Habeeb [1*], Mohammed Hakim Abed Bresam[1] and Eleas M. Wu[2]

[1] *Al-Qasim Green University, College of Agriculture, Department of Animal Production, 51013, Babylon, Iraq*
[2] *Iowa State University, College of Veterinary Medicine, Veterinary Diagnostic and Production Animal Medicine, Ames, Iowa*
[*]*Corresponding author:* Hayder75r@yahoo.com; Hayder.Habeeb@agre.uoqasim.edu.iq; eleaswu@iastate.edu

Abstract

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Ram semen is rich in polyunsaturated free fatty acids which reduces the ability for prolonged semen storage to be used in the assisted reproductive technologies such as artificial insemination. The current research was conducted to evaluate the effect of adding cholecalciferol, or vitamin D3, to chilled Awassi ram semen on Alanine-aminotransferase (ALT) and Aspartate-aminotransferase (AST) enzyme activity, known factors in evaluating sperm membrane damage. Semen samples were collected from three Awassi rams. Vitamin D3 was added to the samples after dividing them into four groups: T1 = 0.02 g/mL; T2 = 0.004 g/mL; T3 = 0.002 g/mL and T0 = control (no additives). Following treatments, samples were cooled to 5ºC and centrifuged at 3000 RPM for 15 min to collect seminal plasma. Seminal plasma was kept under -20°C for later evaluation. ALT and AST concentrations were evaluated at 0 and 72 h following storage. The results showed ALT concentration increased significantly ($P < 0.001$) in T1 samples at 0 hours compared to other treatments. However, the treatment had no significant effect on AST concentrations. A significant decrease in AST was observed at T1 and T2 at 72 h following storage. We conclude a high level of vitamin D3 (T1) might have a detrimental effect on sperm membrane viability but a low level of vitamin D3 (T3) might have a protective effect on sperm membrane viability.

Keywords: Vitamin D3; AST; ALT; semen storage; antioxidant

Introduction

Sheep production is a main sector of agriculture for many countries. Iraq is suffering from reduced animal productivity which may be enhanced with reproductive efficiencies (Al-Barzinji & Othman, 2013) such as advanced reproductive techniques (ART) (Ashrafi et al., 2013). Semen cryopreservation is an ART method used to store sperm for a long period of time. Ram semen is rich in polyunsaturated free fatty acids which reduces the ability for prolonged semen storage to use in assisted reproductive technologies such as artificial insemination (Ax et al., 2000). Alanine-aminotransferase (ALT) and Aspartate-aminotransferase (AST) enzymes are found in sperm heads and used as a parameter to assess sperm membrane damage (Brown et al., 1971). Currently, these enzymes are used as measurements for health issues in mammals such as tissue necrosis (Knudsen et al., 2016), myocardial infarction (Li et al., 2021), white muscle disease (White et al., 2020), and muscular dystrophy (White et al., 2020). Although traditionally evaluated in blood plasma, the activity of ALT and AST enzymes in seminal plasma is 20-fold that of blood plasma (Flipse, 1960). In addition,

both enzymes have a relationship in sperm metabolism and sperm motility which is low in viable ram seminal plasma (Perumal et al., 2013; Ruiz-Pesini et al., 2001). They increased in ram seminal plasma with cryopreservation (Buruiana et al., 1978).

The sperm cell membrane is fragile and requires protection from membrane permeability to prevent sperm cell death (Aitken et al., 2012). Antioxidants are considered a vital source to protect the sperm membrane permeability and prolong sperm storage, such as plants (e.g.ginger, lycobin, black seeds, and thymus) (Al-Malaly et al., 2013; Al-Zubaidy et al., 2013; Habeeb et al., 2020) and vitamins (e.g. A, E, D) (Abdelrahman & Al-karablieh, 2016; Bresm & Habeeb, 2022a) Vitamin D3, or cholecalciferol, is an active form of vitamin D. It has many active physiological roles such as calcium metabolism (Lips, 2006), cell differentiation, and proliferation (Bouillon et al., 1995). Recently, vitamin D deficiency has been shown to affect negatively on male fertility (Blomberg Jensen et al., 2010; de Angelis et al., 2017). It has a role in the control of calcium absorption which is important for hyperactivation, acrosome reaction, and sperm viability (Yoshida et al., 2008).

Our recent finding in sheep concluded vitamin D is a good source of antioxidants to protect stored semen viability for up to 72 h following vitamin D treatment (Bresm & Habeeb, 2022a; 2022b). In bovids, cryopreserved semen supplemented with vitamin D enhanced the bull sperm parameters following the thawing process by protecting the sperm from oxidative damage (Asadpour et al., 2021). Further, vitamin D is found naturally in different areas within the sheep reproductive tract such as the epididymis and seminiferous tubes (Jin et al., 2015). The objective of the current research was to evaluate the effect of adding three different concentrations of vitamin D3 to the chilled Awassi ram semen on the Alanine-aminotransferase (ALT) and Aspartate-aminotransferase (AST) enzymes activity.

Materials and Methods

All research was conducted at the Al-Qasim Green University, Animal Production Department, Babylon, Iraq. Three mature Awassi rams (age range 2-5 years, weight 40-60 kg) were used in this study. The rams were trained for semen collection via an artificial vagina for two months. Samples were collected, pooled and diluted with a tris-egg yolk extender (1:10). Pooled samples were divided into four groups (two samples/treatment) three treatments and one control. Vitamin D3 (SESKRU 99% powder) was added to all treatment groups, except the control group as follows: T1=0.02 g/mL, T2=0.004 g/mL, and T3=0.002 g/mL while T0 had no additives (Bresm & Habeeb, 2022a). Samples were stored in a refrigerator at 5ºC. Seminal plasma was collected by using a centrifuge at 4000 RPM for 30 min at time 0 h and 72 h following treatment and kept at -20ºC until analysis. Alanine-aminotransferase (ALT) and Aspartate-aminotransferase (AST) enzymes were determined by the method described by Karmen et al. (1955). Briefly, woring solution monoreagent (One part of reagents (B) and four parts of reagent (A)) was prepared and kept for 2 weeks at 2-8ºC. One mL of working reagent was added to 100 µL sample and blank tube, mixed and incubate for 1 min at room temperature. Initial absorbance was read against water first then sample at wavelengeth 340 mm. For accuracy, this entire research was repeated six times. One-way analysis of variance repeated measures (ANOVA) was used to evaluate the effect of varying concentrations of vitamin D3 on the Alanine-aminotransferase (ALT) and Aspartate-aminotransferase (AST) enzyme activity (SAS, 2012). To compare the differences between the mean, the Duncan multiple range test was used (Duncan, 1955).

Results and Discussion

The ALT enzyme was significantly lower ($P \leq 0.05$) at 0 h in T2 (4.36±0.57) compared to T0, T1, and T3. However, T1 was significantly greater ($P \leq 0.001$; 19.71±5.25) at 72 h compared to T0, T2, and T3 (Figure 1). It is important to mention T1 increased over time ($P \leq 0.04$; 7.64± 0.69 and 19.71± 5.24) at 0 h and 72 h, respectively (Figure 1). This result was supported by Khalifa (2017) who reported the

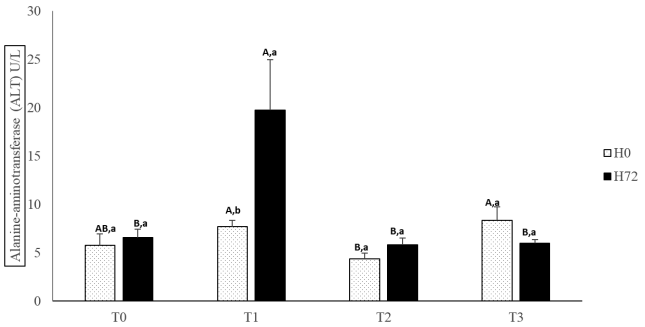

Fig. 1. Effect of three different concentrations of Vitamin D3 to the chilled Awassi ram semen on Alanine-aminotransferase (ALT). Uppercase letters significantly differ ($P \leq 0.05$) between groups within the same time. Lowercase letters significantly differ between times within the same treatment. Two different times (h0 and h72) were evaluated following three different concentrations of vitamin D3 (T0 = control (no treatment), T1 = 0.02, T2 = 0.004, and T3 = 0.002 gm/mL vitamin D3)

additive of antioxidant materials to the ram diluted semen decreased the ALT parameter (Asadpour et al., 2021).

AST was lower at 72 h in the T1 ($P \leq 0.05$; 3.97±0.75) and T2 ($P \leq 0.02$; 4.17±0.89) samples compared to 0 h for T1 and T2 (10.47± 2.83 and 8.82± 1.53), respectively, (Figure 2). However, there was no significant effect of treatments on AST (Figure 2). This result was supported by El-Khawagah et al. (2020) who reported antioxidant additives to the diluted semen decreased the AST parameter following semen storage.

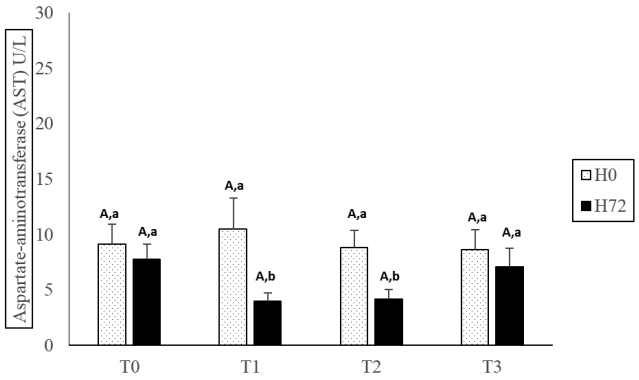

Fig. 2. Effect of three different concentrations of Vitamin D3 to the chilled Awassi ram semen on Aspartate-aminotransferase (AST). Uppercase letters significantly differ ($P \leq 0.05$) between groups within the same time. Lowercase letters significantly differ between times within the same treatment. Two different times (h0 and h72) were evaluated following three different concentrations of vitamin D3 (T0 = control (no treatment), T1 = 0.02, T2 = 0.004, and T3 = 0.002 gm/mL vitamin D3)

Both ALT and AST enzymes are released as a result of sperm membrane damage (Corteel, 1980). They are found in the sperm head and used as a marker of sperm membrane viability (Pesch et al., 2006). Therefore, a high level of intracellular enzymes reveals leakage of the membrane resulting in sperm death. In the current study, a high level of ALT was accompanied by a high concentration of vitamin D3 (T1) which suggests the high concentration caused damage to the sperm membrane and thus spermatic death (Bresm and Habeeb, 2022a). On the other hand, a low level of vitamin D3 (T3) is associated with a low concentration of ALT and AST suggesting the effect of vitamin D3 as an antioxidant (Bresm & Habeeb, 2022b).

Conclusions

The current study suggests vitamin D3 may have a protective effect on chilled storage semen but is concentration dependent. The high concentration of vitamin D3 increased the ALT enzyme parameter but decreased the AST enzyme activity. However, a low level of vitamin D3 decreased the ALT and AST enzymes. These results may indicate a high level of vitamin D3 has a detrimental effect on sperm membrane viability but a low level of vitamin D3 has a protective effect on sperm membrane viability. Further studies are recommended in varying aged males with larger sample sizes.

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