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EFFECT OF THE ADDITION OF GUANIDINOACETIC ACID TO THE DILUTE TRIS IN SOME OF THE BIOCHEMICAL CHARACTERISTICS OF THE SEMEN OF THE RAMS

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Abstract

The study was planned to evaluate the effect of adding Guanidinoacetic acid in some characteristics of the sperm of the rams. This study was conducted in the animal field of the Animal Production Department / Faculty of Agricultural Engineering Sciences / University of Baghdad, for the period from 5/8/2018 to 28/2/2019. In this experiment, 3 rams were used at the age of 2-2.5 years and weighed 50-54 kg. The semen was collected early in the morning and once a week and the semen was pooled to remove the individual differences. The treatments were divided: GAA-free control group, treatment T1 (0.05 mg / 100 ml GAA), T2 treatment (0.1 mg / 100 ml GAA) and T3 treatment (0.2 mg / 100 ml GAA). The results of the study showed a significant decrease in the concentration of malondialdehyde at time (0 and 72 hours) from cryopreservation in treatment T3 compared with the rest of the treatments. There were no significant differences between time-saving coefficients (0) in AST concentration. The AST concentration of time was reduced by 72 hours of conservation in T3 compared to control. ALT concentration was significantly reduced in T3 when conserved by time (0 and 72 hours) of cryopreservation compared with other treatments. The concentration of glutathione significantly increased at time T3 (0 and 72 h) from preservation of cooling. SOD concentration was significantly increased in T3 (0 and 72 hours) of conservation. There was no significant effect of GAA in creatine kinase activity throughout the experiment.

Keywords **:** Guanidinoacetic acid, cryopreservation, Arginine metabolism

Introduction

The addition of Guanidinoacetic acid (GAA) is known as Glycocyamine, which is derived from the reactions of arginine metabolism and glycine in the liver and kidney (Dilger *et al.*, 2013). GAA contributes to the synthesis of creatine (Ct) in S-adenosylmethionine transfer reactions to GAA (Welker, 1979). Creatine is a high-energy phosphatase that is important in the metabolism of the body's energy, especially in the male reproductive system (Schmidt *et al.*, 2004). Vigue and colleagues (1992) report that Ct is responsible for the transfer of ATP energy to the tail of the sperm to provide adequate energy for its motility. Any disturbance in energy metabolism leads to a decrease in sperm motility as this is a key factor for successful fertilization (Etches, 2000). Guanidinoacetic acid is very important for the normal growth and development of embryos, especially the maintenance and energy conservation of tissue growth (Lreland *et al.*, 2009). In addition, it is considered essential in ATP increase and as a result an improvement in fertility due to creatine that increases the sperm motility (Lee *et al.*, 1998). Therefore, it is necessary to find suitable materials for the icing of the rams without affecting the pressure of the azumosity and vitality. Therefore, the aim of the research is to explain the effect of adding guanidinoacetic acid to the tris in some semen characteristics of the ram.

Materials and Methods

This study was conducted in the animal field of the Animal Production Department / collage of Agricultural Engineering Sciences / University of Baghdad for the period from 5/8/2018 to 28/2/2019. The process of collecting sperm from 3 rams by artificial vagina and once a week. The samples were folded and diluted 1:10. The strain dilator was prepared by Salamon and Maxwell (2000) and three concentrations of guanidinoacetic acid, the first treatment (0.05 mg / 100 ml), the second (0.1 mg / 100 ml) and the third 0.2 mg / 100 ml) for diluted semen plus control group. Attributes studied the researchers calculated the concentration according to the method of researchers Guidet and Shah, (1989).Effectiveness of ALT and AST: Effectiveness was estimated by Retman and Frankel (1957) based on the kit prepared by French biomerieux. Glutathione peroxides (GSH-PX): The concentration of the enzyme As Wheeler and others (1990). Superoxide dismutase (SOD): A standard kit manufactured by Sunely Biotechnology Co., Ltd. Creatine kinase (CK): Used a kit kit produced by the Spanish company Biosystems. The Statistical Analysis System (SAS) (2012) was used for data analysis, and the differences between the averages were compared with the Duncan test(1955).

Results

The results of the study showed a significant decrease ($P < 0.01$) at time (0 and 72 hours) from cryopreservation in treatment T3 (0.65 ± 0.02 and 1.89 ± 0.03 µmol / 10^9 sperm) respectively compared with other treatments (Table 1).

Table 1 : Effect of adding different concentration of Guanidinoacetic acid to Tris extender in Malondialdehyde (µ/ 10^9 sperm) at (0 and 72 hrs) of cooling period (5ºC) for Awassi rams semen

Treatment	Time	
	0 hour	72 hour
Control	0.84 ± 0.03 a	2.90 ± 0.05 a
T1	0.73 ± 0.05 a	2.46 ± 0.08 b
T2	0.74 ± 0.03 a	2.38 ± 0.18 b
T3	0.65 ± 0.02 b	1.89 ± 0.03 c
P VALUE	**	**

Different superscripts within column are significantly different (P<0.01) **

C: (Tris), T1: (0.05 mg/100ml), T2:(0.1mg/100ml) and T3: (0.2mg/ 100ml).

The results of the present study indicate that there were no significant differences between the treatments at zero time in AST concentration. The enzyme concentration of T3 was reduced by 72 hours ($P < 0.05$) (60.66 ± 1.76 IU / L) compared with control group. (Table 2). The results showed

a significant decrease (P <0.01) in the concentration of ALT in the T3 treatment at the time (0. 35 ± 1.76 IU / L) concentration compared to the control group (42.66 ± 1.45 IU / L). In cooling preservation after 72 hours, the concentration of the enzyme was significantly reduced (P <0.01) by T3 (80.66 ± 1.33 IU / L) compared with control group (88.33 ± 1.20 IU / L) (Table 3).

Table 2 : Effect of adding different concentration of Guanidinoacetic acid to Tris extender in AST enzyme level (U/L) at (0 and 72 hrs) of cooling period (5ºC) for Awassi rams semen

Treatment	Time	
	0 hour	72 hour
Control	32.33 ± 1.45 a	72.33 ± 1.76 a
T1	32.00 ± 0.57 a	62.00 ± 4.04 b
T2	30.00 ± 1.15 a	61.66 ± 1.20 b
T3	30.88 ± 0.88 a	60.66 ± 1.76 b
P VALUE	N.S	*

Different superscripts within column are significantly different (P<0.0**5**) *, N.S: Non significant

C: (Tris), T1: (0.05 mg/100ml), T2:(0.1mg/100ml) and T3:(0.2mg/ 100ml).

Table 3 : Effect of adding different concentration of Guanidinoacetic acid to Tris extender in ALT enzyme level (U/L) at (0 and 72 hrs) of cooling period (5ºC) for Awassi rams semen

Treatment	Time	
	0 hour	72 hour
Control	42.66 ± 1.45 a	88.33 ± 1.20 a
T1	40.33 ± 0.88 a	86.33 ± 0.88 a
T2	38.33 ± 0.78 b	81.33 ± 1.45 b
T3	35.66 ± 1.76 b	80.66 ± 1.33 b
P VALUE	**	**

Different superscripts within column are significantly different (P<0.01) **

C: (Tris), T1: (0.05 mg/100ml), T2:(0.1mg/100ml) and T3:(0.2mg/ 100ml).

The addition of GAA to the Tris dilute resulted in a significant increase (P <0.01) in the level of glutathione peroxidase for seminal plasma of rams during the time (0 and 72 hours) of cryopreservation as the concentration of the enzyme increased in T3 (120.00 ± 0.57 and 74.33 ± 0.33 U / ML) respectively compared to the treatments (Table 4).

Table 4 : Effect of adding different concentration of Guanidinoacetic acid to Tris extender in GPx enzyme level (U/ML) at (0 and 72 hrs) of cooling period (5ºC) for Awassi rams semen

Treatment	Time	
	0 hour	72 hour
Control	105.00 ± 2.88 b	68.00 ± 0.55 c
T1	104.00 ± 1.52 b	71.00 ± 0.57 b
T2	105.00 ± 0.57 b	72.00 ± 0.57 b
T3	120.00 ± 0.57 a	74.33 ± 0.33 a
P VALUE	**	**

Different superscripts within column are significantly different (P<0.01) **

C: (Tris), T1: (0.05 mg/100ml), T2:(0.1mg/100ml) and T3: (0.2mg/ 100ml).

The results of the present study showed a significant increase (P <0.01) in the concentration of SOD at (0 and 72 h) in T3 treatment (0.57 ± 52.66 and 0.78 ± 37.33 U / ML) (Table 5). The addition of GAA to the Tris dilution had no significant effect on the concentration of creatine kinase enzyme at time (0) of preservation of cooling, , But at time (72 hours) of preservation, T3 was the lowest concentration of creatine kinase (0.35 ± 0.05 IU / L) compared to the control group (0.38 ± 0.08 IU / L) (Table 6).

Table 5 : Effect of adding different concentration of Guanidinoacetic acid to Tris extender in SOD enzyme level (U/ML) at (0 and 72 hrs) of cooling period (5ºC) for Awassi rams semen

Treatment	Time	
	0 hour	72 hour
Control	46.33 ± 0.88 b	28.00 ± 1.15 c
T1	46.66 ± 1.45 b	29.66 ± 0.88 c
T2	47.33 ± 0.88 b	33.33 ± 0.88 b
T3	52.66 ± 0.57 a	37.33 ± 0.78 a
P VALUE	**	**

Different superscripts within column are significantly different (P<0.01) **

C: (Tris), T1: (0.05 mg/100ml), T2:(0.1mg/100ml) and T3:(0.2mg/ 100ml).

Table 6 : Effect of adding different concentration of Guanidinoacetic acid to Tris extender in creatine kinase activity (U/L) at (0 and 72 hrs) of cooling period (5ºC) for Awassi rams semen

Treatment	Time	
	0 hour	72 hour
Control	0.20 ± 0.01	0.38 ± 0.08 a
T1	0.20 ± 0.01	0.38 ± 0.05 a
T2	0.20 ± 0.01	0.36 ± 0.01 c
T3	0.19 ± 0.5	0.35 ± 0.05 b
P VALUE	N.S	**

Different superscripts within column are significantly different (P<0.01) **, N.S: non-significant

C: (Tris) , T1: (0.05 mg/100ml) , T2:(0.1mg/100ml) and T3:(0.2mg/100ml).

Discussion

The reason for the low concentration of malondialdehyde is indicated by the addition of GAA to the Tris dilution, it may be due to the fact that the GAA metabolite leads to the production of arginine and the latter is important in increasing the effectiveness of glutathione. As a result, this enzyme lowers the effectiveness of the malondialdehyde level during the cooling period (Fathi and Tanhn, 2015). The concentration of enzymes ALT and AST was significantly reduced when treated with GAA under study; this is consistent with the conclusion (Dhami *et al.*, 1990) that high AST and ALT may have a negative effect on sperm function. This may be used as an indicator to assess the quality of the sperm. He pointed out that the addition of acid GAA has reduced the level of enzymes in the plasma sperm and this evidence of the preservation of metabolism and protect the sperm from the damage of oxidative stress in the conservation of cooling and thus improve the vitality of the sperm. The results agreed with Al-Daraji *et al.* (2012) that there was no significant effect of adding GAA in liver enzymes. The concentration of the antioxidant enzymes increased significantly at T3, SOD elevation may be due to

the fact that GAA supplementation may be directly or indirectly affected by creatine as an antioxidant (Orita *et al.*, 1978, Wang et al 2012). Thus improving the ability to remove toxicity from H_2O_2 and Superoxide anion, or by increasing total antioxidants and increasing the effectiveness of antioxidant enzymes (Gpx and SOD). This is in line with Lawler *et al.* (2002), who note that the addition of GAA has improved the effectiveness of antioxidant enzymes, including SOD, by 35%. This increase may reduce the damage caused by oxidative stress due to sperm retention for different periods of cooling. The results showed that a significant increase in the level of MDA in the seminal plasma of fertile males was accompanied by elevated serum creatine kinase (Pasqualotto *et al.*, 2001). Agarwal *et al.* (2006) concurred with the fact that oxidative stress was increased in sterile males, resulting in an increase in ROS levels, The high or high creatinine kinase level was observed in sterile males, this result was consistent with Mazzilli *et al.* (1994), the level of creatine is an important indicator of immature sperm and coincides with the rise of malondialdehyde in seminal plasma.

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