Plant Archives Vol. 19, Supplement 2, 2019 pp. 1486-1488 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF DIFFERENT CONCENTRATIONS OF BOVINE SERUM ALBUMIN ON SOME OF THE FROZEN SPERM CHARACTERISTICS OF THE RAMS

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Abstract

This study was conducted in the Faculty of Agricultural Engineering Sciences - University of Baghdad in the field of sheep and goats in the Department of Animal Production. Effect of different concentrations of bovine albumin on some semen characteristics of frozen rams. Used in this experiment, 3 rams and their ages ranging from 2.5-3 years. The sample of the semen was diluted with Tris dilution with the addition of 10 ml of egg yolk per 100 ml, the sample by 1:1 with the tris and then the collection of sperm samples (Pooling) after the semen was divided into four treatments Control and bovine albumin at concentrations of 5, 7.5 and 10% and then complete dilution 10: 1, at 5C, dilution 20: 1, containing 5% glycerol per 100 ml. The results showed that the concentration 5% of bovine albumin showed the best percentage of motility and integrity plasma membrane of the sperm and a significant decrease in dead sperm and mitochondrial apoptosis after thawing.

Keywords: bovine, albumin, frozen sperm

Introduction

The main problem faced by livestock projects in Iraq is the low productivity of one head of sheep due to genetic factors, the spread of diseases and the low level of management and care provided to them (Arab Organization for Agricultural Development, 2001). Small ruminants (sheep and goats) are better suited to the needs of low-income breeders than ruminants, Because of their small size and early maturity and production of meat, milk and fiber and ease of breeding (Galli and Priest, 1984). The freezing of semen for the bulls has been very successful in various countries of the world, including Iraq, but this technique did not achieve the same success for the rams. This may be due to the nature of sperm characterized by high sensitivity to temperature changes during freezing and thawing, high unsaturated fatty acids and low cholesterol in sperm membrane (Salamon and Maxwell, 1995, Bansal and Bilaspuri, 2011). Bovine albumin is a low-density lipoprotein protein of up to 69,000 kt and its atomic capacity is 607 and has solubility in water (Batellier *et al*., 2001). The Bovine albumin contains 17 di-sulphate (S-S) and Sulfhydryl (SH-) (Frank, 1975). One of the most important functions of bovine albumin is the removal of free radicals formed by oxidative stress, as well as its ability to protect the sperm membrane from cracking and rupture during the freezing and thawing of semen (Uysal *et al*., 2005). Bovine albumin is a hyperactivation of the sperm by increasing the flow of calcium ion (Ca^{++}) into the plasma membrane, leading to lower cholesterol and phospholipid of the outer acrosome membrane. This stimulates sperm in the process of capacitation and acrosome reaction (Yamshiro *et al*., 2006). The aim of this study is to use different concentrations of bovine albumin on some of the characteristics of frozen sperm for rams.

Materials and Methods

This study was conducted in the Faculty of Agricultural Engineering Sciences - University of Baghdad in the field of sheep and goats in the Department of Animal Production, for the period from January 2019 to March 2019. Used in this experiment, 3 rams and their ages ranging from 2.5-3 years and body weight ranges from 75 - 80 kg, Using the artificial vagina of sheep and goats, the rams are set up to make a false falsehood to increase their sexual desire (Badawy *et al*., 1975). The sample of the semen was diluted with Tris dilution, and the dilute was prepared according to the method described by Evans and Maxwell (1990) with the addition of 10 ml of egg yolk per 100 ml, After the completion of the combination, the additive is added to the semen gradually and not, to the contrary, to avoid a shock to the sperm to reduce the sample by 1:1 with the tris and then the collection of sperm samples (Pooling) after the semen was divided into four treatments Control and bovine albumin at concentrations of 5, 7.5 and 10% and then complete dilution 10: 1, At 5C, dilution 20: 1, containing 5% glycerol per 100 ml of dilute, is completed and left for two hours to be a period of equilibrium as indicated by Gao *et al*. (1997). The semen is filled in the straws and then transferred to the liquid nitrogen bath. The samples are subjected to nitrogen vapor for 10 minutes in the nitrogen vapor (-75 m) and then immersed in nitrogen liquid (-196 m) and left for one month. After a month of liquid nitrogen freezing, the process of thawing is done by removing the straws from liquid nitrogen and placing it in the water bath at 37C for 5 minutes. The semen characteristics of the individual motility(Chemineau *et al*., 1991), dead sperm(Swanson and Beardon, 1951) and plasma membrane integrity (Jeyendean *et al*., 1984) and the Mitochondrial apoptosis (Ying *et al*., 2001). 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

In Motility sperm, it can be seen that a significant difference between treatments in this trait. The biggest value (35.00 ±0.44%) was recorded from (BSA 5%) which differed significantly from all other treatments. The least value was (28.80±0.37) that came from treatment (BSA 10%) which also differed from all other significantly. No significant differences were seen between the control and treatments (BSA 7.5%) which were recorded (30.40±0.50 and 30.80±0.58) respectively (Figure1).

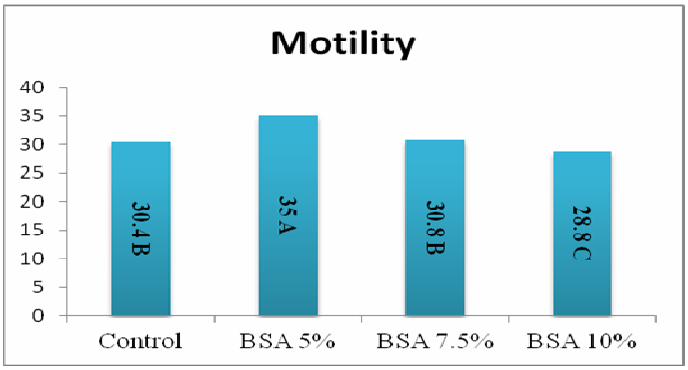

Fig. 1 : Effect of different concentrations of bovine albumin on the individual motility of the sperm after thawing

The integrity plasma membrane illustrates the differences in (HOST) characteristic. It's obvious that the priority was belonged to (BSA 5%) treatment which recorded the highest value (38.80±0.37%) in a significant difference in comparison with the other treatment which were no significant differences among them. The lowest degree (34.00±0.70) recorded by the control treatment. While the other was (34.60±0.50, 33.40±0.50%) for BSA 7.5% and BSA 10% treatments respectively (Figure2).

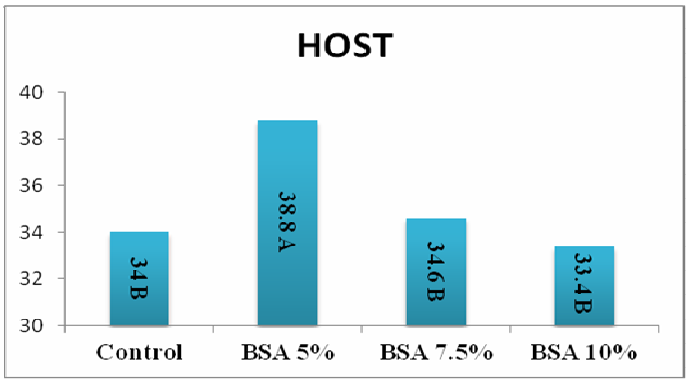

Fig. 2 : Effect of different concentrations of bovine albumin on the integrity plasma membrane of the sperm after thawing

The dead sperm refers to significant differences between (BSA 10%) treatment which had the highest value (46.60±0.50) and all others, while no significant differences were recorded among other three which recorded (46.40±0.50 , 46.20±0.58 , 45.40±0.50%) respectively. The lowest value (44.00±0.70%) was belonging to (BSA 5%) (Figure3). 5% concentration of bovine albumin recorded the lowest percentage of mitochondria apoptosis (75.40±0.50%) Compared with concentrations of 7.5 and 10% and control recorded (77.00±0.70, 79.00±0.70, 76.60±0.50%) (Figure 4).

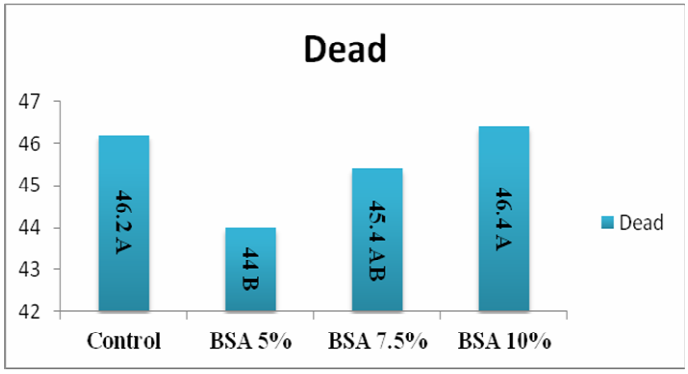

Fig. 3 : Effect of different concentrations of bovine albumin on the dead of the sperm after thawing

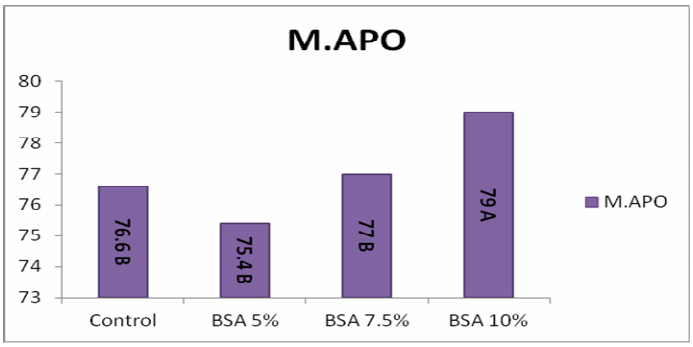

Fig. 4 : Effect of different concentrations of bovine albumin on the mitochondria apoptosis of the sperm after thawing

Discussion

The main purpose of sperm preservation is to prolong the age of sperm from the start of collection of semen until the pollination by reducing the metabolic activities of the sperm by cooling or stopping the metabolic processes for the purpose of keeping the sperm for a longer period without any defect in fertilization by freezing (Holt, 1997 and Manafi, 2011). In this study, non-Penetrating preservatives were used, which cannot be accessed through the plasma membrane, interacting with the membrane and thus having an external effect, namely bovine serum albumin (5, 7.5 and 10%). The results of the study showed that the addition of bovine albumin at 5% concentration of the sperm of the rams resulted in an improvement in semen characteristics after thawing. The percentage of individual motility and the integrity of the plasma membrane of the sperm (35.00 ± 0.44 and 38.00 ± 0.37) respectively. This may be due to several reasons, including the ability of the albumin to bind with the ions (Fe^{++} and Cu^{+}), which stimulates hydroxyl radical (OH *) production, which inhibits the production of unsaturated fatty acid peroxides (Alvarez, Storey, 1983, and Yamashiro, 2006). These results were agreed with Uysal *et al.* (2007) in the ability of bovine albumin concentration (5 mg / ml) to improve the motility of bull sperm after thawing compared with control treatment (58.0 ± 1.3 and 53.0 ± 3.2%). The researchers attributed the cause of a decline in semen characteristics after thawing at high concentrations of albumin to the increase in ammotic pressure of the diluted and its effect on sperm vitality, this view was supported by the researchers (Anghel *et al.*, 2008). The study by Osman *et al.* (2012) differed in the use of albumin at different concentrations (0, 4, 8, 12, 16 mg / mL) in the semen dilution of bulls, noting that the concentration of 8 mg / ml was superior to the rest of the concentrations. In several studies, it has been observed that effective oxygen classes inhibit the work of enzymes important for energy production in oxidative phosphorylation and glycolysis, Decreased energy production in mitochondria affecting sperm motility (Twigg *et al.*, 1998; Kao *et al.*, 2008). Holt (1997) confirmed the association between the progressive motility of sperm rams and mitochondria in semen cooling, noting that a decrease in sperm motility was associated with mitochondrial dysfunction due to the cooling process. It is important to note that the process of freezing and thawing limits the activity of antioxidants, making sperm more susceptible to free radicals and accelerating the triggering of apoptosis and damage to the DNA (Lasso *et al.*, 1994; Wang *et al.*, 2003). By using the concentration of 5% of the bovine albumin has improved the characteristics of sperm thawing in the rams.

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