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Study on the Effect of Adding Bovine Serum Albumin to Semen Diluent on the Viability of Awassi Ram Semen Preserved at 5ºC

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Abstract

This study was aimed to test the effect of adding bovine serum albumin (BSA) to Tris-fructose-egg yolk diluent to increase the ram sperm storage period at 5ºC. Semen samples from six mature Awassi rams used in this study. Semen samples were diluted by Tris-glucose-egg yolk. Diluted semen sample was divided into two parts. The first part BSA was added 10 mg ml-1 and the second part considered as a control group without any addition. The diluted semen samples were cooled and preserved at 5ºC for 5 days. Cooled diluted semen samples were examined for individual motility, percent of live sperm, sperm abnormalities and acrosomal defects every 24 h until 5 days. Results of the present study showed an increased viability of spermatozoa diluted in the Tris diluent containing BSA stored at 5ºC for 120 h of preservation compared with the control group. Spermatozoa motility and viability declined gradually in BSA Tris containing diluent when preserved at 5ºC from 0 h to 120 h to 54.0±0.9% and 59.0±0.9 %, respectively compared to control of Tris diluent without BSA which was declined markedly to 35.9±1.9 %, 40.9±1.8 %, respectively. In conclusion, this study demonstrated that supplementation with BSA of ram semen diluents during semen preservation at 5ºC, may exert beneficial effects on the quality of the Awassi ram semen.

Keywords: Awassi ram, Semen, Dilution, Tris, Bovine serum albumin

Introduction

Semen diluents are of critical importance in avoiding the loss of viability due to the dilution, cooling and storage of sperm cell. Sperm cells have a high content of unsaturated fatty acids in their membranes and they lack a significant cytoplasmic component containing antioxidants. Therefore, sperm cells are highly susceptible to lipid peroxidation by free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl radical, which lead to the structural damage of sperm membranes during the aerobic storage of sperm (Sinha *et al.,* 1996). The cooling and aerobic preservation processes produce physical and chemical stress on the sperm membrane which sequentially reduces sperm viability and fertilizing ability. Cold shock of sperm cells during the cooling process is associated with oxidative stress induced by free radicals (Sanacka and Kurpisz, 2004). Free radicals are mostly eliminated by antioxidant systems. Antioxidants play an important role in scavenging free radicals which may cause lipid peroxidation of sperm plasma membranes (Baumber *et al*., 2000). The addition of antioxidants is well known to improve the viability and motility of liquid storage or cryopreserved ram sperm cells (Maxwell and Stojanov 1996, Baumber *et al,*. 2005). One of the most important features of bovine serum albumin (BSA) is known as the elimination of free radicals generated by oxidative stress, and the protection of membrane integrity of sperm cells from cold shock to eliminate free radicals generated by oxidative reactions, and therefore to protect the membrane integrity of sperm cells from lipid peroxidation during the semen freezing process (Lewis *et al.*, 1997). Several authors (Matsuoka *et al.*, 2006, Uysal and Bucak, 2007, Kubovicova *et al*., 2010, Barati *et al*., 2011) reported that BSA can be substituted for egg-yolk in the ram semen diluent, and that it enhances the motility and viability of bull and ram sperm cells following the freezing-thawing process. The aim of this study was to test the effect of adding bovine serum albumin to Tris-fructose-egg yolk diluent to increase the ram sperm storage period at 5ºC.

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Materials and methods

Animals and semen collection

Semen samples from six mature Awassi rams (2-3 years of age) used in this study, and maintained using conventional feeding, housing and lighting conditions. The study was carried out from September 2010 to June 2011. Animals were housed at the Animal Research and Practice Farm of the College of Veterinary Medicine, University of Mosul, Mosul at 36°20' N, 43° 8' E. All these rams were in good health. They were maintained in identical nutritional and managerial condition throughout the period of study. Throughout the experimental period, the animals were kept in open front barrens, were fed individually with concentrated mixture of 1 kg per ram per day, and were given water ad libitum. A total number of 60 ejaculates were collected from the rams using an artificial vagina once a week. For collecting ejaculates, rams were penned with ewes in estrus, in the presence of a handler with an artificial vagina. Ejaculates were evaluated and accepted to include in this study, if the following criteria were met: volume varying between 0.5-2 ml; sperm concentration of 1-2 x 10^9 sperm/ml; the motile sperms percentage higher than 70% and less than 10% abnormal sperm in total.

Semen analysis

The volume of each ejaculate was recorded and sperm concentration was determined using semen diluted with 3% NaCl, the diluted semen was placed on a hemocytometer with the sperm counted in five squares of one chamber. Sperm motility was identified as those sperm cells that demonstrated progressive motility. Sperm motility was scored from zero to 100% by a qualified and experienced investigator. Semen was placed on a heated glass slide, and scoring was performed at microscopic magnification of 200 ×. Each sample was evaluated twice. The mean value was used for data analysis. Assessment of abnormal and normal spermatozoa was performed using an eosin–nigrosin staining method (Salisbury *et al*., 1978). For the percent of spermatozoa with abnormal acrosomes, fast green stain was used (Wells and Awa, 1970).

Dilution of semen and addition of BSA

Semen samples were diluted by Tris-glucose-egg yolk (Hydroxy methyl amino methane-Tris 3.6 g, glucose 0.5 g, citric acid 1.7 g, streptomycin sulphate 100 mg, crystalline penicillin 100000 IU, double distilled water 85 ml and egg yolk 15 ml). Semen quality was re-evaluated to ensure that the dilution has not affected the semen quality. The diluted semen sample was divided into 2 parts. The first part BSA (Sigma Chemical Co., St Louis, MO, USA) was added 10 mg ml-1 and the second part considered as a control group without any addition. The diluted semen samples were cooled gradually and preserved at 5°C for 5 days. Cooled diluted semen samples were examined for individual motility, percent of live sperm, sperm abnormalities and acrosomal defects every 24 h until 5 days.

Statistical analysis

Data for the percentages of motility, live sperm, abnormal sperm and acrosomal defects were arcsine transformed and statistical analyses were performed with the software (Sigma stat, Jandel scientific software V2.0, Richmond, CA, 2004). Data were expressed as mean (±S.E.). Variance of homogeneity of samples was examined by Levene's test. The differences between mean of the same parameter were tested by the student t test.

Results

In this experiment no differences (P >0.05) were found between rams in the evaluated parameters. Ram effect was eliminated from the model. Results of the present study showed an increased viability of spermatozoa diluted in the Tris diluent containing BSA stored at 5°C for 120 h of preservation compared with the control group. The effect of adding BSA to Awassi ram semen diluted with Tris on sperm characteristics following the preservation at 5°C for 120 h are presented in Table 1. Spermatozoa motility and viability declined gradually in BSA Tris containing diluent when preserved at 5°C from 0 h to 120 h to 54.0±0.9% and 59.0±0.9 %, respectively compared to control of Tris diluent without BSA which was declined markedly to 35.9±1.9 %, 40.9±1.8 %, respectively. There were significant (P < 0.05) main effects of BSA addition to semen diluent on sperm motility as well as the sperm viability in different times of preservation at 5°C. Significantly (P < 0.05) higher sperm abnor-

malities and acrosomal defects values (37.6±1.3 % and 71.5±1.1 %, respectively) were found after 120 h incubation in Tris free BSA (control) at 5°C compared with those obtained in Tris diluent containing BSA (22.3±0.8% and 57.5±1.4 %, respectively).

Table1. Mean (± SEM) values of individual motility, live sperm, abnormal sperm, and abnormal acrosomes of Awassi ram semen diluted and stored at 5°C for 120h of preservation with Tris diluent containing BSA and control group

	Individual motility (%)	live sperm (%)	Abnormal sperm (%)	Abnormal acrosomes (%)
Tris with BSA	54.0 ± 0.9^{a}	59.0 ± 0.9	22.3 ± 0.8^{a}	57.5 ± 1.4^{a}
Control	35.9 ± 1.9^{b}	40.9 ± 1.8^{b}	37.6 ± 1.3^{b}	71.5 ± 1.1^{b}

Mean for each parameter in the same row, with different superscript (a, b) are significantly different *(P*<0.05).

Discussion

The effect adding BSA to ram semen diluents on semen quality has been studied recently (Maxwell and Stojanov 1996, Baumber *et al*., 2005, Uysal and Bucak, 2007, Kubovicova *et al*., 2010, Barati et al., 2011). This study is the first to report the effect of adding bovine serum albumin to Tris-fructose-egg yolk diluent to increase the ram sperm storage period at 5ºC of Awassi rams in Iraq. The results of the present study showed that diluted Awassi rams semen with Tris diluent containing10 mg ml-1 of BSA improve sperm motility and viability, and to protect acrosome and membrane integrity. Our results showed that sperm motility and viability could be maintained and improved at 120 h of preservation at 5°C. Post-preservation sperm motility (54%), morphologic sperm abnormalities (22.3%), acrosome damage (57.5%), and viable sperm (59%) were determined for 120 h of storage at 5oC in this study, which are close to the findings of Kubovicova *et al*. (2010) and Barati *et al.* (2011). Sperm cells are highly susceptible to lipid peroxidation by free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl radical, which lead to the structural damage of sperm membranes during the dilution-cooling-storage process (Sanacka and Kurpisz, 2004). BSA can protect membrane integrity of sperm cells from heat shock during dilution-cooling-storage process of spermatozoa (Lewis *et al.*, 1997, Uysal *et al*., 2005). A probable improvement in semen quality by addition of BSA in ram semen diluent is more likely related to an inhibition of lipid peroxidation of the sperm plasma membrane as was revealed by Kubovicova *et al*. (2010) and Barati *et al.* (2011). In conclusion, this study demonstrated that supplementation with BSA of ram semen diluents during semen preservation at 5°C, may exert beneficial effects on the quality of the Awassi ram semen. This study has shown that many aspects of sperm protection, e.g. sperm motility, viability and membrane stabilization of sperm cells during preservation at 5°C are of prime importance to BSA.

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