

# Effects of Different Plant Extracts on Post-Thaw some Spermatological Parameters of Cryopreserved Awassi Rams Spermatozoa

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## Abstract

Several diluting-protecting media and different methods have been used for storage of ram semen in liquid and frozen state (Salamonand Maxwell, 2000). Addition of preservatives media for increasing storage of sperm are very important for successful artificial insemination in rams. This study was conducted on evaluate the effect of 3 plant extract. Ejaculates were collected using an electro-ejaculator twice a week during there productive season. After pooling, each pooled ejaculate was split into four equal aliquots and diluted with based Tris, Citric acid, Egg yolk, glicerol extender containing plant extracts (0.02% and % 0.04 Rosemary (*Rosmarinus officinalis*) (Rosemary extract -oil-soluble)); Echinacea (*Echinacea purpurea*) (0.5 mg/mL and 1 mg/ml) and St. John's wort (*Herba Hyperici*) (0.5 mg/mL and 1 mg/mL) at a final concentration of approximately  $2 \times 10^8$  motil spermatozoa per straw. These results indicate that that the addition of Echinaceaand Rosemary as antioxidants have more positive effects for Awassi rams on spermatological parameters.

**Keywords:** *plants, cryopreservation, Awassi rams*

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## INTRODUCTION

The Awassi is the most numerous and widespread breed of sheep in south-west Asia (Figure 1). The processing and storage of ram semen reduce the motility and disrupt the membrane integrity of spermatozoa. It is generally assumed that these changes are detrimental and are associated with a loss of fertility capacity. Despite many years of empirical research that has established a variety of methods for the processing, storage and insemination of spermatozoa, fertility is generally lower after cervical insemination with stored than fresh semen (Maxwell, and Watson, 1996).

It has been shown that sperm quality during freeze-thawing process can be better maintained

by addition of various antioxidants to semen extender compared to control (Bilodeau *et al.*, 2001). There are limited document studying the effect of plant extracts as a antioxidants on sperm quality.

Ram sperm are extremely sensitive to oxidative stress due to the high content of unsaturated fatty acids present in the plasma membrane phospholipids in comparison to other species (Alvarez, and Storey, 1983; Alvarez *et al.*, 1987). Many studies indicate that the quality of semen deteriorates during liquid storage for a long period (Kasimanickam *et al.*, 2007). Seminal plasma confers some protection against ROS damage via glutathione (GSH), catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide-dismutase



**Figure 1.** Awassi Rams

(SOD) (Hammadeh *et al.*, 2009). Nonetheless, the protective role of the seminal plasma is reduced after applying several dilutions for semen storage (Martínez-Páramo *et al.*, 2009). To cope with such problems, different antioxidants have extensively been added to the extenders to improve ram sperm quality (Bucak, and Tekin, 2007). Different natural herbs have been studied for the several antioxidant properties. These herbs contain many phytochemicals (carotenoids, polyphenol, flavonoids) widely used in cosmetic, pharmaceutical and food industry with beneficial effects (Zheng, and Wang, 2001). Recently, there has been a global trend concerning natural antioxidants that are present in fruits, vegetables, plants, oil seeds, and herbs to preserve semen quality (Del Valle *et al.*, 2013; Motlaghet *et al.*, 2014).

Therefore, the present study was conducted to determine the effects of some plants added to extender of Awassi rams semen on some spermatological parameters after thawing of cryopreserved semen.

## **MATERIALS AND METHODS.**

### *Reagents*

Unless otherwise stated, reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA). Plant extracts were purchased from Immu-Nat Herbal Medicine and Natural Health Products (Mugla, Turkey).

### *Semen Collection and Evaluation*

Ejaculate samples obtained from three mature rams in this study. Ejaculates were collected using an electro-ejaculator twice a week during their productive season. After pooling, each pooled ejaculate was split into four equal aliquots and diluted with based Tris, Citric acid, Egg yolk,

glycerol extender containing plant extracts (0.02% and 0.04% Rosemary (*Rosmarinus officinalis*) (Rosemary extract -oil-soluble); Echinacea (*Echinacea purpurea*) (0.5 mg/mL and 1 mg/mL) and St. John's wort (*Herba Hyperici*) (0.5 mg/mL and 1 mg/mL)) at a final concentration of approximately  $2 \times 10^8$  motile spermatozoa per straw. Sperm motility was determined subjectively using light microscope (Olympus, Japan) at 400 magnification. Sperm density was determined according to the haemocytometric method. For this aim, sperm was diluted at ratio of 1:500 with Hayem solution (5 g  $\text{Na}_2\text{SO}_4$ , 1 g NaCl, 0.5 g  $\text{HgCl}_2$ , 200 ml bicine) and density was determined using a 100 ml Thoma haemocytometer (TH-100, Hecht-Assistent, Sondheim, Germany) at 400 x magnification with Olympus BX50 phase contrast microscope (Olympus, Japan) and expressed as spermatozoa  $10^9/\text{ml}$ . Sperm counting chambers were always kept in a moist atmosphere for at least 10 min. Before cell counting. Sperm pH was measured using indicator papers (Merck, 5.5–9) within 30 min of sampling. To assess live/dead sperm percentage, eosin-nigrosin preparations were made according to the method noted by Björndahl *et al.*, (2003). Totally 300 sperm cells were counted on each slide at 1000 x magnification. Spermatozoa with abnormal morphology were assessed by the Hancock's solution (HS) (HS: 62.5 ml formalin, 150 ml sodium saline solution, 150 ml buffer solution, and 500 ml double-distilled water). For this assessment, three drops of semen suspension were mixed with 1 ml HS. Afterward, one drop of this mixture was placed on a slide and mounted with a coverslip. The percent of abnormal spermatozoa was recorded by counting 200 spermatozoa under phase contrast microscopy at 1000 x. The hypo-osmotic swelling (HOS) test was

**Table 1.** Effect of plants on post-thaw motility, viability, abnormal rates of cryopreserved Awassi ram semen

Plants	Plants Concentrations	Post-thaw Motility (%)±S.D.	Viabile Cells (%)±S.D.	Abnormal Rates (%)±S.D.	Host (%)±S.D.
Echinacea	0.5 mg/mL	53.7±3.7 <sup>a</sup>	48.1 ± 1.6 <sup>a</sup>	23.4±1.3 <sup>a</sup>	40.5±1.3 <sup>a</sup>
	1 mg/mL	50.2±2.3 <sup>a</sup>	59.2 ± 1.3 <sup>b</sup>	22.3±1.4 <sup>a</sup>	31.3±2.7 <sup>b</sup>
Rosemary	0.02%	48.2±1.4 <sup>b</sup>	56.2±1.4 <sup>b</sup>	23.8±1.2 <sup>a</sup>	38.0±1.4 <sup>a</sup>
	0.04%	43.2±1.1 <sup>c</sup>	59.6 ± 1.5 <sup>b</sup>	25.6±1.5 <sup>a</sup>	32.5±1.7 <sup>b</sup>
St. John's wort	0.06 mg/mL	42.4±1.5 <sup>c</sup>	60.5 ± 1.9 <sup>b</sup>	23.3±1.4 <sup>a</sup>	30.2±1.2 <sup>b</sup>
	1 mg/mL	33.6±1.6 <sup>d</sup>	68.2 ± 3.4 <sup>c</sup>	24.6±1.2 <sup>a</sup>	29.6±2.1 <sup>b</sup>
Control	--	36.5±2.1 <sup>d</sup>	65.9±2.4 <sup>c</sup>	27.6±1.3 <sup>a</sup>	24.9±1.4 <sup>c</sup>

used to evaluate the functional plasma membrane of spermatozoa as described by Revell and Mrode (1994). Briefly, 10 µl semen were incubated with 100 µl hypo-osmotic solution (9 g/l fructose and 4.9 g/l sodium citrate, 100 m Osm/kg) at 37 °C for 60 min. Afterward, 0.1 ml of the mixture was placed on a microscope slide and mounted with a coverslip. A total of 400 spermatozoa were evaluated and sperm with swollen and coiled tails were determined in each sample under phase-contrast microscopy at 400x.

#### Statistical Analysis

Results are presented as mean±SE. Data for percentage of sperm motility and fertilization were transformed by angular transformation prior to statistical analysis by SPSS 22.0 software. Differences between parameters were analyzed by repeated analysis of variance (ANOVA). Significant means were subjected to a multiple comparison test (Duncan) at level of  $\alpha = 0.05$ .

### RESULTS AND DISCUSSIONS

Post-thawed spermatozoa motility had significant difference at the groups with plants Echinacea (0,5 mg) 53,7±3,7% and rosemary (0,02 %) 48,2±1,2%, compared to the control (36,5±2,1%). At abnormal spermatozoarates, while there was not statistically difference among the groups. Dead spermatozoa with the lowest rate was attained at group with Echinacea. (0,5 mg) At Host, Echinacea and rosemary gave the highest rates (40,5±2,7% and 38,0±1,4%,  $P < 0,05$ ), statistically (Table 1).

In this study Echinacea, rosemary and st. john's wort extracts were used for inhibiting detrimental effects of freeze-thawing process on ram semen. The results of the present study demonstrated that treatment with Echinacea and rosemary extract can efficiently change the total and motility, viability, and membrane integrity. Our result about motility agree with the result of other studies Malo *et al.*, (2011) and Zhao *et al.*, (2009).

### CONCLUSIONS

In conclusion, it was seen that some plant extract (Echinacea and rosemary) added to extender of Awassi rams semen provided the significant contributions on post-thawing some spermatological parameters.

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