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THE EFFECTS OF ADDITION OF OMEGA-3 FATTY ACIDS ON SOME QUALITY OF AWASSI RAM'S CHILLED SPERM

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Abstract

This study was carried out in Department of animal Reproduction, College of Agriculture, university of Diyala to investigate the effect of adding omega$_3$ to tris extender on the some semen characteristics Color and Volume of ejaculate, individual motility, live sperm, abnormal sperm%, Viability of sperm Acrosome integrity%, ALT and AST to 3-5 day after storage at (5℃). Semen was collected from 3 Ram a 1.5 to 2 years old and 46-53kg body weight. All Semen in the treatments pooled and divided into four parts adding different concentratAwassi of omega$_3$ to the tris extender (T0=0, T1=0.5, T2=1, T3 =2 %) from percentage of egg yolk in extender. The results showed that there were significant differences (P≤0.05) in individual motility between (T2,T3) comparison to (T0,T1) to Third day after storage at 5℃. And in live sperm percentage comparison to T0 at Third day, there was significant differences (P≤0.05) in live sperm percentage between (T2) comparison to (T0,T1) at fivth day. There was non significant differences (P≤0.05) between treatment in abnormality sperm percentage. The results showed that there were significant differences (P≤0.05) in Acrosome integrity between (T2,T3) comparison to (T0,T1) to Third day. The results showed that there were dicrease significant differences (P≤0.05) in AST, ALT between (T2,T3) comparison to (T0,T1) to Third day after dilution and storage at 5℃.

Keyword: Storage of semen, Omega-3, Ram

Introduction

Sheep are important farm animals, so improving their reproductive performance is important to increase their productivity and then increase their numbers (AL-Haboby and colleagues, 2003). Improving the reproductive efficiency of rams is by increasing sperm that can reach the fertilization site depending on the quality and quantity of the semen produced (Saacke, 1994). The choice of a suitable dilator is an important step in the success of artificial insemination in small ruminants (Abdelhakeam *et al*., 1978), the damage of metabolic oxidation processes that naturally occur in semen on sperm is a major cause of the production of fatty acid peroxides, a key factor in reducing sperm movement and loss of fertilization ability (Jones, Mannt, 1977; Ball *et al*., 2001) Specific interferes with the reproduction of animals Agricultural data (Wu *et al*., 1979 and Kotowska and Kotowski, 2001 and Baiomy *et al*., 2009) to avoid the problem was Many efforts have been made to improve sperm preservation of the rams by refrigeration by maintaining the sperm movement and the integrity of the plasma membrane by adding antioxidants to the thinners (Watson, Anderson, 1983). The essential fatty acids of omica type are an important requirement for maintaining normal formation, cellular membrane functAwassi and enzymatic activity (March, 1992; Ibeas *et al*., 1994), as well as the production of hormone-like substances (Karendmand *et al*.; 2002) Which mediate physiological processes of metabolism and activity as processes of muscles and nerves (Stillwell and Wassall, 2003).

Material and Methods

Semen collection: The rams were trained on the process of collecting semen for 25 days before the experiment. The semen was collected from 3 rams using an artificial vagina at 41 ℃, one time / week in the morning, while the place and date of collection remained constant. The experiment was used as a urn for the rams during the collection process and allowed the rams to make false falsehoods to increase their sexual desire (Badawy *et al*., 1975). The objective is to study the effect of reducing the seminal fluid by reducing the gear containing Omega3,6,9 and refrigerating it with 5 m and 3 Consecutive days to determine the best transact Awassi in maintaining the vitality of the sperm for as long as possible.

Design of the Experiment

- control T0: Dissolve semen without adding Omega3.
- T1: Omega3 was added by 0.5 ml / 100 ml gear dampener.
- T2: Omega3 was added by 1 ml / 100 ml gear dampener.
- Third treatment T3: Omega3 was added by 2 ml / 100 ml of the gear dampener

Preparation of the dilator: Preparation of the gear according to the method of Salamon and Maxwell (2000) agencies:

Table 1 : Gear attenuation components

Component	Conc.
Tris (hydroxymethyl amino methane) (CH_2OH_3) CNH_2 molecular weight 121.14	3.63g\100ml
Citric acid monohyrdate ($C_6H_8O_7.H_2O$).	1.99g\100ml
Glucose	0.50 g\ 100ml
Osmotic pressure	300 mOsm
Egg yolk (Fresh unfertilized eggs)	10ml\100ml
pH	7.0
Penicilline	100.000 IU\100ml
Streptomycin	100mg\100ml

Evaluation of Semen

The sperm collection tube is transferred directly to the laboratory to measure the size of the shell. It is placed in the water bath at 37 °C, and then the following tests are done:

- **Ejaculate volume:** The volume of the Ejaculate recorded immediately after collection by used a glass tube.
- **Individual Movement:** Estimated by Walton Method (1933)
- **Dead Sperm Ratio:** Dead sperm calculated according to the method of Swanon and Bearden (1951).
- **Mutilated Sperm Ratio:** calculated according to the method of Hancock (1951).
- **HSOT:** Hyperosmotic swinging test

The percentage of sperm with the proper plasma membrane was estimated by Delgadillo and colleagues (1992) with 10.0µg of test tube semen with a hypo-osmotic solution (fructose 8.72 g / L and Sodium citrate 4.74 g / L) with osmosic pressure (100 mOsm / L) And (PH) (8.00), and placed in a water bath for (60 minutes) at a temperature (37 m), after the end of the incubation period take a drop and put it on a glass slide temperature (37 m), with the cover slide They were examined under a microscope with a magnification force (400X). The sperm were counted in different fields of the slide, after which the percentage of the swollen and tailed tail was calculated.

Measurement of the concentration of the enzymes of the group of the Secretary of the: - At the end of each week of the experiment collected semen from all animals of the transaction is the isolation of plasma semen with centrifugation (Centrifugation) and was kept plasma semen for the purpose of conducting chemical tests, which included measuring the concentration of enzymes The glutamic oxalo acetic transaminase (GOT) and glutamic pyruvic transaminase (GPT).

These enzymes were evaluated according to the instructAwassi of Biomaghrtta, which is equipped with Kit

Statistical analysis of data:

The data were analyzed using the Completely Randomized Design using the following mathematical model and using SAS program (SAS, 2000). The Duncan Multidisciplinary Test (1955) used to test the differences between the averages.

$$Yij = \mu + Ti + eij$$

Results and Discussion

Evaluation of Sperm Characteristics of Local Rams:

The results showed that the ejaculation color of all rams was creamy and there were no significant differences ($P < 0.05$) in the size of the shell of the rams under study. The mean was 1.27 ± 0.11. The results of the study showed significant differences ($P < 0.05$) the average overall movement of all rams was 81.03 ± 1.74, compared with the ram (2), while not significantly different ($P < 0.05$) compared to ram 1. The results of the study were also shown, but there were individual differences between the rams in both the percentage of dead sperm as well as For the percentage of distorted sperm but did not reach the level of morbidity (Table 2).

That the different treatments had a significant effect ($P < 0.05$) in maintaining the individual movement of the quail preserved at 5°C and for 72 hours after dilution compared to the a control treatment (Table 3). ($P<0.05$) in the ability to preserve the semen with a higher degree of cooling compared with other treatments. This moral superiority continued until 72 hours after dilution. Due to the ability of Omega3 to increase the plasma membrane's fluidity, which improves its motor mobility, the membrane's membrane fluid increases the membrane's susceptibility to damage caused by change in the oxidative pressure of Maldjian *et al*., 2005, the percentage of live sperm decreased with the percentage of dead sperm of the total Compared to control group during The periods of the experiment may come back Omega3 as it works to protect the sperm of the active oxygen (ROS), which is one of the products of oxidation and lead to the damage of the plasma membrane of the sperm because it contains a high proportion of polyunsaturated fatty acids, as the Omega-3 adhere to the installation of the plasma membrane, Reactive oxygen (ROS) Omega3 contains a high proportion of polyunsaturated fatty acids (El Darawany, 1999).

As for the effect of the addition of Omega3 on the integrity of the plasma membrane of the sperm, there was a significant increase (P00.05) for T2 and T3 when compared with the T0 and T1 treatment the day after cryopreservation at 5 °C (Table 4). The difference in the percentage of the plasma membrane of the sperm may be due to the difference in the osmotic pressure of the added treatments Omega3 because of the increase in sperm movement and survival, i.e., the increase in the percentage of the live and the decrease in the percentage of dead sperm Differences in sperm movement and plasma membrane integrity during addition of Omega3 are due to the ability of Omega3 to increase the plasma membrane's fluidity, which improves its motor viability. The plasma membrane's fluidity increases the membrane's ability to resist damage caused by changes in nitrogen pressure (Yimer *et al*., 2014; Maldjian *et al*., 2005). The results showed that there was a significant decrease (P 0.05) for AST concentration for T2 and T3, respectively, compared to T0 and T1, respectively, as well as ALT concentration in the treatment compared with T0 and T1 respectively on the third day of refrigeration (Table 4). The low concentration of AST and ALT may be attributed to the low percentage of mutant and dead sperm with low membrane permeability of the semen cell, which causes the enzymes not to be released to the sperm plasma. Foote, 1999, demonstrates the vitality of the sperm El-Darawany, 1999. The high level of active oxygen The main reason for the damage of the genetic material and the disruption of the outer and internal membrane function of the sperm and thus the triggers of programmed death, especially C-Cytochrome and Caspase, we conclude that the addition of the levels of Omega3 1% and 2% of the reduction of the gear reduced the cooling damage to the vitality of local Awassi.

Table 2 : General characteristics of the seminal fluid of the rams under study (average ± standard error)**:**

Treatments	No. of observation	Ejaculated vol. ml	Individual Movement	Dead sperm (%)	Abnormal sperm (%)
T0	6	1.23±0.05[a]	84.94±1.50[a]	9.63±0.92[a]	4.47±0.74[a]
T 1	6	1.28 ±0.06[a]	80.68 ±1.13[b]	9.92±1.18[a]	4.71±0.82[a]
T 2	6	1.30±0.09[a]	85.47± 1.40[a]	9.59±1.30[a]	4.45±0.52[a]
T 3	18	1.27±0.11	83.69±1.74	9.71±1.21	4.54±0.52

The averages with different letters within the same column differ significantly at (P <0.05)

Table 3 : Effect of Omega3 on individual movement of sperm after dilution and cryopreservation (mean ± standard error)

Time from dilution (hours)	No. of observation	T0 0%	T1 0.5%	T2 1%	T3 2%
before diluted	6	79.31±0.54a	80.30±0.21a	79.88±0.67a	80.00±0.87a
after diluted 0	6	75.64±0.82a	76.43±0.65a	76.52±0.61a	77.64±0.82a
24\	6	65.21±1.76c	67.21±1.35b	70.73±1.06a	69.43±1.20ab
48	6	62.84±1.60c	64.84±1.60b	68.64±1.17a	65.60±1.41b
72	6	59.87±1.48c	61.51±0.93bc	65.06±1.06a	63.01±1.32b

The averages with different letters within the same column differ significantly at (P <0.05)

Table 4 : Effect of adding Omega3 in sperm characteristics after 3 days of cooling (mean ± standard Error)

Treatments	Individual Movement %	HSOT %	AST U/L	ALT U/L
T0	58.43± 1.12 b	62.43± 1.12 b	35.81± 0.13 a	34.27± 0.31 a
T 1	59.78± 1.24 b	65.42± 1.30 b	33.50± 1.22 a	32.24± 1.25 a
T 2	64.73± 1.23 a	71.23± 0.13 a	23.57± 0.15 b	24.32± 0.17 b
T 3	62.52± 1.30 a	74.31± 1.17 a	24.75± 1.16 b	23.87± 0.21 b

The averages with different letters within the same column differ significantly at (P <0.05)

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