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Effect Of Alcoholic Extract Of Opuntia Ficus Indica On Semen Quality In Post-Thawed Of Awassi Rams.

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ABSTRACT

The present study was aimed to test the efficacy of adding different concentration of alcohols extract of Opuntia ficus indica fruits(AEOFI) to Tris fructose egg yolk diluents to enhance semen quality on post – thawing motility, plasma membrane integrity and dead sperm of Awasii rams.This experiment was executed at the farm Animal – Department of Animal Production – College of Agriculture – University of Baghdad AL-Jadreya campus during the period from the first October, 2017 to 27 th of march, 2018. The observed result in experiment after one month post-thawing showed that the Individual motility of sperm were improved significantly ($p<0.05$) by used 1% of (AEOFI) in the extender which was (32.75±0.85%) compared to the control group (26.75±0.62%), also after tow month post-thawing it was significantly increases in 1% concentration of (AEOFI) which was(32.00±0.70%) compared to the control group ((25.75±0.47%). The data also showed that the extender containing 1% (AEOFI) concentration was significantly ($p<0.05$) better than other concentration of (AEOFI) for the membrane integrity of sperm. The percentage of dead sperm decreased significantly ($p<0.05$) by the 1% (AEOFI) concentration better than other group. In conclusion, the result of presented study indicated that the 1% concentration of (AEOFI) better than the other concentration for freezing of Awasii rams sperm.

Keyword: Sperm, Awasii ram, Post-thawing, Opuntia ficus indica.

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INTRODUCTION

The cryopreservation is a complex technique (Purdy, 2006). It is mostly accepted that a main number of sperm are deteriorate during cryopreservation (Watson, 2000). This deteriorate may lower sperm motility and increases dead sperm and the fertilization rate after artificial insemination (Matsuoka et al, 2006). Damage during cryopreservation has been caused by cold shock or ice crystal formation or toxicity or oxidative stress or change osmotic pressure (Watson and Martin, 1975).Therefore, the texture of extender, appropriate cryoprotectants and excellent freezing and thawing are very for successful semen freezing (Hammerstedt et al, 1990). Today, herbal medicine is witnessing an exciting renaissance in Western countries due to the limited side effects of this type of treatment, The herb is a low-cost, low-cost source (Kamboj, 2000).Sheep constitute a large part of this livestock in Iraq, which amounted to 6.780 million head and constitute a major source of income for the population of the pastoral areas in Iraq ,Where sheep contribute to filling a large part of the citizens' needs of protein, as the consumer prefers the meat and milk of these animals to a large extent (FAO, 2003).The sperm plasma gives some protection from free radical damage through its antioxidants such as catalase, glutathione peroxidase and superoxide dismutase (Hammadeh et al., 2009) However, the role of sperm plasma, but the process of dilution of semen reduces this role (Martínez-Páramo et al, 2009). Recent researchers have been using natural antioxidants in fruits, vegetables and oilseeds to enhance the vitality of semen (Del Valle et al., 2013).Opuntia ficus-indica are a plant rich in natural antioxidants that are effective in maintaining the motility, vitality and integrity DNA during the freezing process (Meamar et al., 2012).The compounds are tocopherol, polyphenol, flavonoid, phenolic acids, minerals and sulfur amino acids (Stintzing et al., 2001; Feugang et al, 2006).The objective of this study was to investigate the effect of the alcoholic extract of the Opuntia ficus-indica in different concentrations (0, 0.5, 1, 1.5, 3%) to the ram semen extender and on the characteristics of sperm after freezing and thawing.

MATERIALS AND METHODS

Animal and semen collection: Semen samples from three Awasii rams (2-3 years of age and 52-60 kg of Wight) were hired in this study. The rams were housed and fed traditionally. The study was carried out from the first October, 2017 to 27 th of march, 2018. Animals were housed at the farm Animal – Department of Animal Production – College of Agriculture – University of Baghdad, Iraq. All the rams were in a good health. They were safeguard in equal managerial and nutritional situation throughout the periods of the study. The animals were kept in open front barrens, were fed singly. Rams were fed twice a day with a diet with concentrated mixture(Containing 46% barley , 36%bran , 16% soybean , and 2% minerals and salt mixed in the farm) of one kg per ram per day . They had free access to fresh water. Thirty ejaculates were collected by artificial vagina (41-42˚C) during ten weeks. For collecting ejaculates, ram were penned with ewes in estrus. Ejaculates were evaluated and included in this study if the following norm were met, volume of (0.9-1) mL, sperm Concentration of (2.5 χ 10^9)sperm per mL, motility (> 70%) .

Semen analysis: Within 2-3 min after collection the semen kept in a water bath at 37˚C. The volume of each ejaculates were recorded, sperm cells were counted in five squares of a hymocytometer after 1:200 dilution of semen by taken (0.1 m L) semen and diluted with (19.9 m L) diluents solution, formed from(0.9%) Nacl, 0.01 Hgcl and 2 gm/L eosin stain(Salisbury et al, 1943).The assessment of froze-thawed sperm individual motility (%) a sample of the diluted sperm was placed on a heated glass slid, and put a cover slip in the center of a pre-warmed slid (37 ˚C) and it was transferred to the microscope (400x magnification), the percentage of the individual motility was determined as the method described by (Chemineau et al, 1991). Assessment of the percentages of dead sperm was performed using an eosin – nigrosin staining method described by(Swanon and Bearden, 1951), 10µL of diluted sperm mixture with 10µL eosin-nigrosin stains was smeared on glass slide and let the slid to dry, 200 sperm from different field were examined under a microscope 400x. Assessment of the percentage of Sperms plasma membrane integrity was performed using (Lomeo and Giambersio, 1991) method , a semen sample 30 µL was mixed with 300 µL distilled water (pH 7.2, mOsm 0⁄5) and incubated at 37 ºC for 5 min , after incubation a drop 15 µL of the treated mixture was placed on a warmed slid and covered with a cover slip, a total of 200 sperm was counted in six different microscopic fields at 400 x , the percentage of spermatozoa with swollen and curved tails was recorded.

Alcoholic extract preparation. The Alcoholic extract was prepared according to (Zhang et al, 2008) procedure. The dried fruits powder (5g) was mixed with 100 mL of alcohol/water (70:30 v/v). The AEOFI was posterior

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extracted by magnetic stirrer with hot plate for 24 h. the solution was filtered by filter paper. And then but the solution in an oven at 37 ˚C until dried . And stored at 4 ˚C until use.

Dilution of semen and addition of AEOFI: Semen samples were diluted by using the basic extenders were Tris 3.63%, fructose 0.5%, citric acid 1.9%, streptomycin 100 mg, penicillin 100000IU, glycerol 7%, and egg yolk 19.2 mL. The diluted semen sample was divided into five group. The first group Tris egg yolk only without any addition considered as control group, The other four treatment group were adding the alcoholic extract of the Opuntia ficus indica fruits with different percentage which were 0.5% , 1% , 1.5% , 3% for T1 , T2 , T3 , T4 respectively. The extended semen was then packaged in French straw (0.25m L), one end of the straws was closed the other open end of the straws was closed by used the polyvinyl chloride powder. Straws were but in the refrigerator at 5˚C for 3 h before exposed to the vapour of the liquid nitrogen after that but the straws about 4-5 centimeter from the liquid nitrogen surface level about 10 min. They were then submerged in liquid nitrogen for one and tow month. The straws were thawed in water bath at 37˚C about two min. Semen samples were examined for individual sperm motility , plasma membrane integrity, and dead sperm after one and two moth post-cryopreservation.

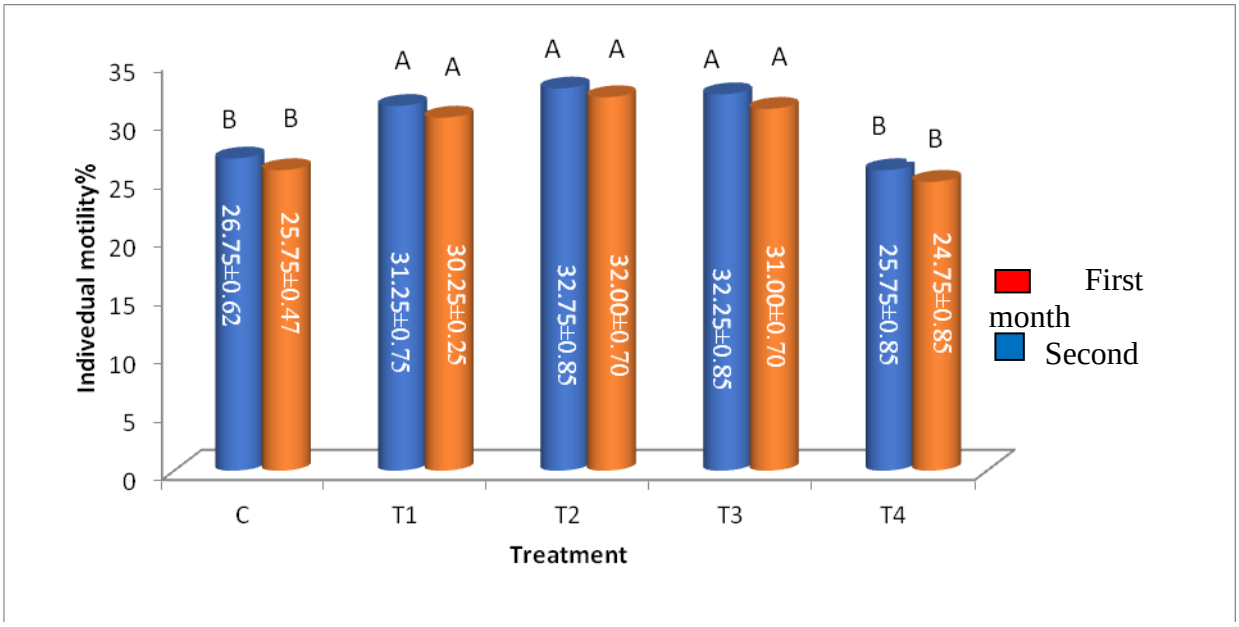
Statistical analysis. Data were analyzed by SAS (2012) computer program using completely randomized design (C.R.D)to study the effect of treatment for any time according to model:

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

Where, Yij is the value of observation j to treatment i, μ the general mean of the characteristic, Ti effect of treatment i, eij : random error which is naturally distributed at an average of zero and a variation of σ^2e. Duncan test was used to compare the significant differences between means

RESULT

Tris egg yolk (TEY) based extenders were added with different concentration of AEOFI (0, 0.5, 1, 1.5, 3%) and sperm quality was investigated at one and two month of frozen. The sperm individual motility did not differ among one and tow month after freezing and thawing .However, the result of our study showed a significant differences ($p<0.05$) were fond in 1% AEOFI which was(32.75±0.85%) compare to the control group (26.75±0.62%) in the individual motility after one month post-thawing (Figure 1). Also Figure 1 shows the effect of 1% concentration was a significant differences ($p<0.05$) in the individual motility after two month post-thawing which was (32.00±0.70%)compare to the control group (25.75±0.47%)



Figure(1): Effect of added different concentration of (AEOFI)to(TEY) on sperm individual motility (%) in post –thawed semen of Awasii rams.

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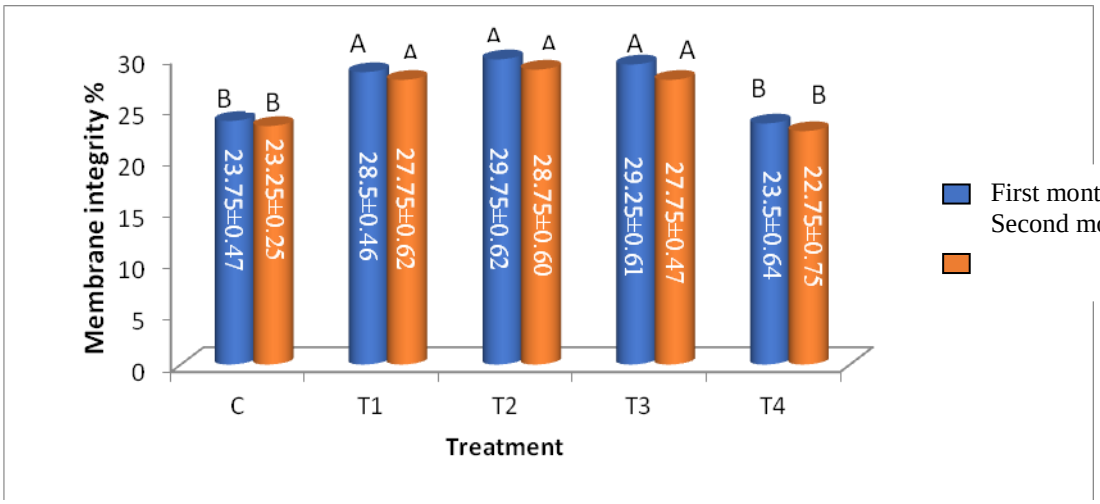
(TEY): Tris egg yolk, AEOFI: alcohols extract of Opuntia ficus indica fruits, C: Control group TEY only, T1: TEY + 0.5% AEOFI, T2: TEY + 1% AEOFI, T3: TEY +1.5% AEOFI, T4: TEY + 3% AEOFI, Values are expressed as mean ± SEM, Means carrying different big superscript letters significantly varied at *p<0.05.

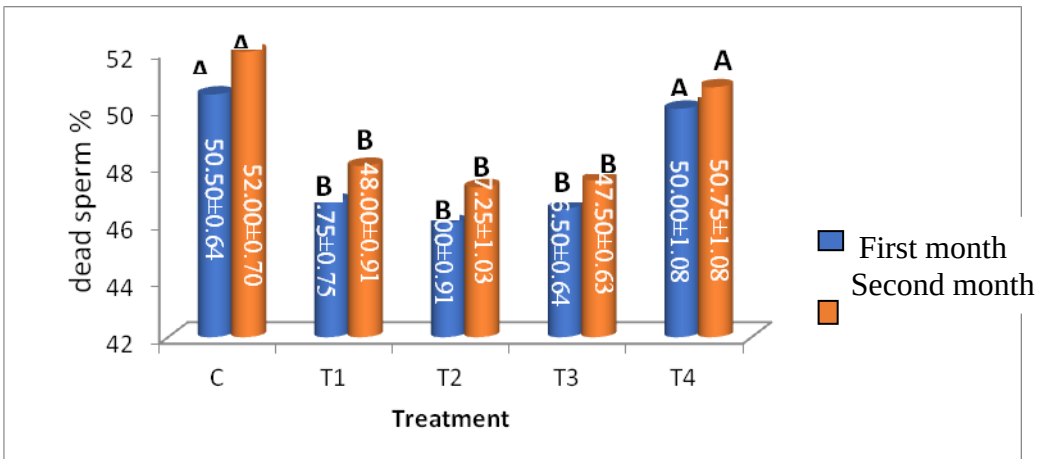
Figure 2 shows the plasma membrane integrity of spermatozoa did not differ among one and tow month after freezing and thawing. Particularly for the combination of TEY supplemented with AEOFI at 1% which was (29.75±0.62%) maintain better plasma membrane compared to control group (23.75±0.47%) after one month post- thawed. Also the same think after two month post-thawed.



Figure(2): Effect of added different concentration of (AEOFI)to(TEY) on plasma membrane integrity (%) in post –thawed semen of Awasii rams.

(TEY): Tris egg yolk, AEOFI: alcohols extract of Opuntia ficus indica fruits, C: Control group TEY only, T1: TEY + 0.5% AEOFI, T2: TEY + 1% AEOFI, T3: TEY +1.5% AEOFI, T4: TEY + 3% AEOFI, Values are expressed as mean ± SEM, Means carrying different big superscript letters significantly varied at *p<0.05.

Figure 3 shows the dead of spermatozoa did not differ among one and tow month after freezing and thawing. The obtained results showed that TEY supplement with 1% AEOFI significantly (p<0.05) lower in post-thawed semen extender in dead sperm which was (46.00±0.91%) compared to the control group (50.50±0.64%) after one month. Also the same think after two month.



Figure(3): Effect of added different concentration of (AEOFI)to(TEY) on dead sperm (%) in post –thawed semen of Awasii rams.

(TEY): Tris egg yolk, AEOFI: alcohols extract of Opuntia ficus indica fruits, C: Control group TEY only, T1: TEY + 0.5% AEOFI, T2: TEY + 1% AEOFI, T3: TEY +1.5% AEOFI, T4: TEY + 3% AEOFI, Values are expressed as mean ± SEM, Means carrying different big superscript letters significantly varied at $^{*}p<0.05$.

DISCUSSION

Scientific research and statistical studies have confirmed that phenol is one of the most widespread secondary metabolites in the plant kingdom. The positive effect of these plants may be partially attributed to the presence of these compounds (Bravo et al., 2013). Flavonoids represent the largest part of this group and have effective anti-oxidant effects. They also have positive effects on health. One of its most important effects is its ability to stabilize cellular membranes and thereby reduce the effect of fatty acid peroxides (Chaudhari et al., 2008). According to many studies, Opuntia ficus-indica contain a wide variety of antioxidant activity such as polyphenols, especially flavonoids, proanthocyanidins, vitamin C, E, β-carotene, clotathione, taurine, and amino acids, Methionine and arginine (Tesoriere et al., 2005; Betancourt-Domínguez et al., 2006). The results of this study showed a significant effect (P <0.05) with a concentration of (1%) of the AEOFI for the individual movement of the sperm and did not differ significantly from the concentration (0.5%) at one and two months after freezing Compared to control group for the individual motility of the sperm. One of the reasons for the decline of sperm motility is changes in the plasma membrane during the stages of conservation and the most important is the exit of enzymes, including the enzyme Glucose-6-phosphate dehydrogenase, which leads to a decrease in the production of ATP and increase the rate of AMP (ADP) Indicates the ability of sucrose added to the diluted semen to maintain the enzymatic efficacy of the sperm (Manafi, 2011). The percentage of sperm motility was reduced after dissolving. The control group was recorded during the first and second months which was (26.75±0.62% and 25.75±0.47%) respectively, and did not differ significantly from the concentration of 3%wich was (25.75±0.85% and 24.75±0.85%) respectively. The percentage of sperm motility was reduced after dissolving, a number of studies indicate the role of effective oxygen classes in inhibiting the work of enzymes important for the production of energy in the processes of Oxidative Phosphorylation and Glycolysis and then decrease the production of energy in mitochondria affecting the movement of sperm (Twigg et al., 1998 and Kao et al., 2008). The stages of the freezing process lead to mechanical stress of the plasma membrane of the sperm and the loss of the Osmotic system, resulting in a change in the amount of water inside the semen (Hammerstedt et al., 1990).The use of concentrations of 3 and 1.5% of alcohols extract of Opuntia ficus indica fruits(AEOFI) and control group resulted in the lowest percentage of plasma membrane integrity. The addition of other natural herbs such as syphilis (Syzygium aromaticum) and rosemary (Rosmarinus officinalis L) improve the quality of semen (Baghshahi et al., 2014; Motlagh et al., 2014). In the study by Allai et al. (2016) noted that the alcohols extract of Opuntia ficus indica improved the percentage of the integrity of the erythematic membrane by reducing the production of fatty acid peroxides. The results of our study agreed with Allai's, (2016). The high percentage of phenolic compounds in the alcoholic extracts of the Opuntia ficus-indica tend to be a good antioxidant (Chougui et al., 2013). The positive effect of the extract of the Opuntia ficus-indica in this study is due to the oxidative action of many components such as quinic acid, malic acid, rutin, hyperoside, and quercitin, as well as Allai et al. (2016).

In conclusion, using Tris fructose egg-yolk-citric extender containing a 1% concentration of (AEOFI) alcohols extract of Opuntia ficus indica fruits during cryopreservation of Ram semen showed the highest sperm individual motility, plasma membrane integrity and lowest dead of spermatozoa in post-thawed semen.

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