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EFFECT OF ADDING DIFFERENT LEVELS OF AMLA FRUIT EXTRACT AND VITAMIN C TO TRIS-BASED EXTENDER IN THE SEMEN PROPERTIES PRESERVED AT 5^{O}C OF AWASSI RAMS

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Abstract

This experiment was carried out in the field for the Animal Production Department belonged to the College of Agricultural Engineering Sciences/ University of Baghdad, from 10 September 2018 to 20 February 2019. In this study, Four local Awassi rams were used with ages ranging from 2-3 years and a weight of 50-58 kg, while the semen was collected weekly *via* artificial vagina. The study aimed to compare the effect of adding a different levels of the aqueous Amla fruit extract as a natural antioxidants and different level of vitamin C (industrial antioxidants) to Tris- based extender in the semen properties that cryopreservation of the Awassi rams. The Experiment treatments were divided into nine treatments that included control treatment C and the 1st treatment T1 with an addition of a 0.3% of the Amla extract and the 2nd treatment T2 with a 0.6% of the Amla extract. Moreover, the 3rd treatment T3 with an addition of 5 mM of vitamin C and the 4th treatment T4 with a10 mM of vitamin C. Furthermore, the 5th treatment T5 with an addition of 0.3% of Amla extract and 5 mM of vitamin C and the 6th treatment T6 with a 0.3 % of Amla extract and 10 mM of vitamin C. Finally, the 7th treatment T7 with an addition of 0.6 % of Amla extract and 5 mM of vitamin C and the 8th treatment T8 added 0.6 % of Amla extract and 10 mM from vitamin C to semen extender. Semen samples were preserved after three days of extender at a temperature of 5^{o}C, where microscopic tests are carried out on semen extender samples that included the individual motility and live sperm percentages for all treatments. The results of the study showed that the addition of the aqueous Amla fruit extract with vitamin C within the T8 group led to a significant increase ($P<0.01$) in the percentages of the individual motility and live sperm compared to the control group during all cryopreservation periods. The individual motility percentage of T8 treatment at the time of 0 of cryopreservation was (93.14 ±0.508%) compared to the control group of (84.57 ±0.719%). While at the time of the 72 hrs of preserved duration, the percentage of sperm individual motility was (67.42 ±1.130%) compared to treatments percentages (C, T1,T2, T3, T4, T5, T6, T7) which were (47.28 ±0.865, 57.14 ±1.280, 59.13 ±0.55, 54.85 ±1.223, 55.85 ±1.010, 62.71 ±0.968, 63.85 ±1.056, 66.00 ±1.023%) respectively. Finally, the live sperm results of T8 were higher than the rest of treatments at the time 48 and 72 of the cryopreservation, which reached (76.71 ±0.606%) and (66.00. ±0.845%) respectively, compared to the control group of (61.14 ±0.737%) and (52.71 ±0.968%) for both periods respectively. It can be concluded that the addition of aqueous Amla fruit extract only or with other types of artificial antioxidants to the Tris- based extender has been instrumental in improving the semen qualities of the Awassi rams after different periods of cryopreservation.

Key words: Amla Fruit, Vitamin C, Semen.

Introduction

The first attempts to preserve the sperms at low temperature back to 1776, when the Italian physiologist scientist Ladzard Spalanasi (Spallanazi) stated that the horse freezing sperms in the snow became inactive but can be revived after heating. However, the first resulting method of cryopreservation back to 1950 (Rama *et al*., 2006). The purpose of the sperm preserved process is to prolong its fertilizing ability, while keeping the sperms liquid for a short time is achieved by reducing the food metabolism of sperms by lowering the storage temperature. Furthermore, keeping sperm cryopreservation for a long time is done by reducing the food metabolism of sperms through storage at a temperature below zero centigrade, as well as, keeping semen for a long time at low temperature has helped to prolong sperm life by slowing down the metabolism process in addition to stimulating bacterial growth (Anghel *et al*., 2010). The

World Health Organization (WHO) in 2002, indicated that 80% of the population of the developing countries and 65% of the population of the advancing countries depend on traditional medicine that uses medicinal plants. These types of medicines represent about 25% of total medicines in the United States of America, while its represent about 80% in the countries with fast progress, such as India and China, which consider an importance factor for the economy in these countries. The world's known plant number are 250,000 plants and about 80,000 plants are classified as medical (Borris, 1996), one of these medicinal plants is the Amla plant (*Emblica officinalis*), which is generally spread in almost all Asian countries and it is considered as one of the most important medicinal plants in the Indian medical system (Dasaroju and Gottumukkala, 2014). Amla is also a rich source of vitamin C, as well as a good content of amino acids and minerals (Agarwal *et al*., 2012; Gaire and Subedi, 2014), the aqueous of the Amla plant extract also contains a high percentage of antioxidants and flavonoids (Khan, 2009). (Dutta and Saha, 2013) pointed out that the male mice, administration by the Amla fruit extract has led to an improvement in the average weights of the male genitals, an increasing in the number of sperms, reducing in the number of abnormal sperm, as well as, an improvement in the level of testosterone hormone and reduced the toxic effect of the Chlorpyrifos for administrating to the male mice. Vitamin C or Ascorbic acid, is a water-dissolved vitamins, which is important for the continuation of the body's functions normally and is closely related to fertility (Allai *et al*., 2018). Vitamin C is also important in maintaining the genotype integrity of the sperms and preventing oxidative damage to the sperms DNA (Fraga *et al*., 1991). (Azawi and Hussein, 2013) showed an improvement in the motility percentage and sperm vitality of the Awassi rams that vitamin C was added to it and cryopreservation. (Mittal *et al*., 2014) also pointed out that the addition of vitamin C to the semen extender of bulls has led to a significant decrease in the abnormal sperm percentage and improved both the sperms motility and the percentage of acrosomes safety. Therefore, this study was aimed to compare the effect of adding different levels of Amla extract (natural antioxidants) and different levels of vitamin C (industrial antioxidants) to Tris- based extender in the semen properties stored at 5°C of the Awassi rams.

Materials and Methods

This study was conducted in the Animal Field and the Reproductive Physiology Lab and the Central Laboratory for Postgraduate studies-College of Agricultural Engineering Sciences-University of Baghdad. The experiment extended from September, 2018 to February, 2019 with the aim of comparing the addition of different levels of the Amla fruit extract (natural antioxidant) and different levels of vitamin C (industrial antioxidants) to Tris-based extender in the semen properties stored at 5°C of the Awassi rams. Four local Awassi rams were used with ages ranging from 2-3 years and a weight of 50-58 kg, while the semen was collected weekly *via* artificial vagina. The Experiment treatments were divided into nine treatments that included control treatment C and the 1st treatment T1 with an addition of a 0.3% of the Amla extract and the 2nd treatment T2 with a 0.6% of the Amla extract. Moreover, the 3rd treatment T3 with an addition of 5 mM of vitamin C and the 4th treatment T4 with a10 mM of vitamin C. Furthermore, the 5th treatment T5 with an addition of 0.3% of Amla extract and 5 mM of vitamin C and the 6th treatment T6 with a 0.3% of Amla extract and 10 mM of vitamin C. Finally, the 7th treatment T7 with an addition of 0.6% of Amla extract and 5 mM of vitamin C and the 8th treatment T8 added 0.6% of Amla extract and 10 mM from vitamin C to semen extender. Semen samples were preserved after three days of extender at a temperature of 5°C, where microscopic tests are carried out on semen extender samples.

• *Sperm evaluation*: The individual motility of sperm was estimated according to (Walton, 1933) and the living sperm were calculated based on (Swanson *et al*., 1951) method.

• *Statistical Analysis*: Statistical Analysis System SAS, (2012) was used to analyze the data to study the effect of treatments and the time on the studied traits according to a completely randomized design (CRD). The significant differences between the averages were compared to the Duncan's test, (1955).

Results and Discussion

Individual motility of sperm

Table 1, indicates the effect of adding different levels of vitamin C and Amla extract and their mixture in the percentage of individual sperm motility when preserved at 5°C, for the Awassi rams. Table 1 shows that there are high significant differences ($P<0.01$) in the individual motility of sperm at the periods (48 and 72 hours) from cryopreservation, where the treatments (T7, T8) were superior at 72 hours with a ratio of (67.42 and 66.00%) respectively, compared to Amla fruit added treatments (T1, T2) with a ratio of (57.14 and 59.13%) respectively and vitamin C added treatments (T3, T4) with a ratio of (54.85 and 55.85%) respectively.

Table 1: The effect of different levels of Amla fruit extract, vitamin C and cryopreservation duration at 5°C on the individual motility percentage (%) of the Awassi rams sperms (average ±standard error).

Treatments	Time 0	Time 24 hrs.	Time 48 hrs.	Time 72 hrs	Significant level
C	84.57 ± 0.719D a	74.42 ± 1.151C b	62.00 ± 1.704D c	47.28 ± 0.865F d	**
T1	88.57 ± 0.685C a	81.00 ± 1.362B b	70.42 ± 1.269C c	57.14 ± 1.280DE d	**
T2	90.28 ± 0.918D a	81.42 ± 1.630B b	72.00 ± 1.023CB c	59.13 ± 0.55D d	**
T3	84.71 ± 1.248D a	75.71 ± 1.686C b	64.71 ± 1.768D c	54.85 ± 1.223E d	**
T4	86.00 ± 0.975D a	75.71 ± 1.745C b	65.00 ± 1.214D c	55.85 ± 1.010E d	**
T5	89.28 ± 0.521BC a	82.57 ± 1.087AB b	72.71 ± 1,304ABC c	62.71 ± 0.968C d	**
T6	91.57 ± 0.782AB a	82.57 ± 0.895AB b	73.00 ± 1.290ABC c	63.85 ± 1.056BC d	**
T7	91.57 ± 1.172AB a	84.28 ± 0.918AB b	74.57 ± 1.136AB c	66.00 ± 1.023AB d	**
T8	93.14 ± 0.508A a	86.00 ± 0.816A b	76.28 ± 0.837A c	67.42 ± 1.130A d	**
Significant level	**	**	**	**	

Notes:- Significant (P<0.01) **
Means with large letters within a column indicate a comparison between treatments.
Means with small letters within a row indicate a comparison between the different periods within the same treatment.

This superiority in individual motility of sperm can explain from the fact that aqueous extracts of the medicinal plants containing a phenolic compounds, which can prevent fat oxidation in cell membranes (Radwan Nadiia *et al.,* 2008). Where the appearance of Reactive Oxygen Species (ROS) during cryopreservation has reduced oxygen that associated with the fat oxidation in the sperm membranes that destroy the chemical structure of fat in the cell membranes, this damage to the fat structure leads to reduced sperm's ability to motility (Bucaket *et al*., 2010). The addition of phenols to the extender leads to protect cellular components from oxidized free radicals (El.Nekeety *et al.,* 2011). These results were also agreed with (Rani *et al*., 2002) findings, where the addition 5 mM of vitamin C to extender led to a high significant improvement (P<0.01) in the percentage of individual sperm motility, which reached (70.42±0.22) compared to the control group (38.70±0.235%). The results of (Osama and Elias, 2013) showed that the addition of 0.9 mg from vitamin C to the extender caused an improvement in the individual motility sperms by a percentage of (54.7±1.1) compared to the control group that was (35.9±1.9), where vitamin C is considered anti-oxidant and eliminates the toxicity resulting from fat oxidation. (Schwenke and Behr, 1998).

Viability of sperms

Table 2, shows the effect of using the Amla fruit extract and vitamin C on the vitality Awassi rams sperms in at 72,48.24.0 hours, where the results showed a high significant difference (P<0.01) between the control treatment and the Amla and vitamin C added treatments. At the 72-hour period, both T1 and T2 treatments which amounted 59.42% exceeded on the control treatment that reached 52.71%. Furthermore, the mixture treatments T7, T8 were exceeded on T5, T6 treatments, while the best treatment among all treatments was T8 by (66.00%)

Table 2: The effect of different levels of Amla fruit extract, vitamin C and cryopreservation duration at 5°C in the live sperms percentage (%) for Awassi rams semen (average ±standard error).

Treatments	Time 0	Time 24	Time 48	Time 72	Significant level
C	85.57 ± 0.812E a	0.799 ± 73.85 b C	61.14 ± 0.737E c	52.71 ± 0.968D d	**
T1	88.42± 0.812a CD	81.00 ± 1.112B b	69.57 ± 0.895BC c	59.42 ± 0.812C d	**
T2	89.28 ± 0.746DC a	82.14 ± 1.454AB b	70.57 1.377±BC c	59.42 ± 1.342C d	**
T3	89.28 ± 0.911DC a	75.57 ± 1.888C b	65.57 ± 1.900D c	54.85 ± 1.261D d	**
T4	87.00 ± 0.690DE a	± 76.28 1.475C b	65.57 ± 1.757D c	55.57 ± 1.461D d	**
T5	89.28 ± 0.808CD a	82.57 ± 1.087AB b	72.42 ± 1.288BC c	61.14 ± 0.73BC d	**
T6	90.42 ± 0.895CB a	82.85 ± 0.911AB b	71.57 ± 2.102BC c	61.28 ± 1.960B d	**
T7	91.71 ± 0.837AB a	83.71 ± 0.746AB b	74.57 ± 0.782AB c	64.42 ± 0.719A d	**
T8	93.28 ± 0.808A a	85.57 ± 0.782A b	76.71 ± 0.606A c	66.00 ± 0.845A d	**
Significant level	**	**	**	**	

Notes:- Significant (P<0.01) **
Means with large letters within a column indicate a comparison between treatments.
Means with small letters within a row indicate a comparison between the different periods within the same treatment.

that significantly exceeded (P<0.01) on the control treatment that recorded 52.71% for the preservation periods 72-hour. Although it is a useful technique to protect sperm by refrigeration, but it can affect the sperm quality such as their ability to survive. This damage can be avoided by the compounds that derived from medicinal plants and their ability to improve sperm quality (Zribi *et al*., 2012). This explains the results that obtained in the number of live sperm, where this study showed a high significant superiority (P<0.01) in the number of live sperm in the treatments which Amla and vitamin C were added to it, as well as for the treatments which Amla extract was added alone, that reflect the effective role of the Amla extract in increasing the number of live sperm. (Saddiki *et al*., 2017) found through their researches that the use of 5°C or 10 µg/ml of phenols increases the live sperm percentage after freezing, where the studies have shown that vitamin C is very important in protecting sperm and this may be due to the ability of vitamin C to prevent the formation of hydrogen peroxide (Askasi *et al*., 1994). These results can be explained by the formation of free radicals that caused a damage to the plasma membrane and thus the necessary enzymes are lost in the metabolism, so that no energy is generated and cause a sperms death (Rizal *et al*., 2003). The addition of vitamin C and phenols can protect the plasma membrane for sperms from damage and the vitamin C acts as an antioxidant that can destroy the formed free radical chains (Herdis *et al*., 2010). Phenols increase sperms survival by inhibiting sugar analysis (Janh *et al*., 2017), which is present a good agreement with what (Poonam and Charu, 2014) obtained when analyzing the Alma fruit, chemically, they found that the vitamin C percentage in the Amla was 630.31mg/100 g and the percentage of phenols in Amla by 25.62 g, which indicates the important role of the Amla in reducing the action of free radicals in our study.

Reference

Agarwal, M., A. Kumar, R. Gupta and S. Upadhyaya (2012). Extraction of polyphenol, flavonoid from *Emblica officinalis, Citrus limon, Cucumis sativus* and evaluation *of Their antioxidant activity*., **28(2):** 993-998.

Allai, L., A. Benmoula, B. Nasser and B. El Amiri (2018). Supplementation of ram semen extender to improve seminal quality and fertility rate, *Anim Reprod Sci*., **192:** 6-17.

Anghel, A. and S. Zamfirescu (2010). Role of antioxidant additives in the protection of the cryopreserved semen against free radicals. *Romanian Biotech. Letters.*, **15(3):** 33-41.

Askari, H.A., J.H. Check, N. Peymer and A. Bollendorf (1994). Effect of NaturalAntioxidants Tocopherol and Ascorbic Acids in Maintenance of Sperm Activity During Freeze-Thaw Process, *Archives of Andrology.*, **33(1):** 11-15.

Bucak, M.N., P.B. Tuncer, S. Sariözkan, N. Başpinar, M. Taşpinar, K. Çoyan, A. Bilgili, P.P. Akalin, S. Büyükleblebici, S. Aydos and S. Ilgaz (2010). Effects of antioxidants on post-thawed bovine sperm and oxidative stress parameters: antioxidants protect DNA integrity against cryodamage. *Cryobiology*., **61:** 248-253.

Burri, B. and R. Jacob (1997). Human metabolism and the requirement for vitamin C.

Dasaroju, S. and K.M. Gottumukkala (2014). Current Trends in the Research of *Emblica officinalis* (Amla): A Pharmacological Perspective, *International Journal of Pharmaceutical Sciences.*, **24(2):** 150-159.

Duncan, D.B. (1955). Multiple range and multiple F. Tests. *Biometrics*., **11:** 1-42.

Dutta, A.L. and C.R. Sahu (2013). Pirotective effect of emblica officinlis on chlorpyrifos can organophate insecticide induced male reproductive system in rat. *Int. J. pharm. Bio. Sci*., **4(1):** 49-58.

El-Nekeety, A.A., S.R. Mohamed, A.S. Hathout, N.S. Hassan, S.E. Aly and M.A. Abdel-Wahhab (2011). Antioxidant properties of *Thymus vulgaris* oil against aflatoxin-induce oxidative stress in male rats. *Toxicon*., **57:** 984-991.

Fraga, C.D., P.A. Motchnik, M.K. Shinegana, H.J. Helbock, R.A. Jacob and B.N. Ames (1991). Ascorbic acid protects against oxidative DNA damage in human sperm. *Proc. Natl. Acad. Sci*., **88:** 11003-11006.

Gaire, B.P. and L. Subedi (2014). Phytochemistry, Pharmacology and Medicinal Properties of *Phyllanthus emblica* Linn. *The Chinese Journal of Integrated Traditional and Western Medicine*., 406-799.

Herdis, I., M. Kusuma, M. Surachman, I.K. Riza, I. Sutama, B.P. Inounu and I. Arifiantini (2010). Improvement of frozen semen quality of Garut Sheep through the addition of alpha-tocopherol into yolk egg-skim milk diluents. *Journal Ilmu Ternak dan Veteriner.*, **7 (1):** 12-17.

Jang, T.H., S.C. Park, J.H. Yang, J.Y. Kim and J.H. Seok (2017). Cryopreservation and its clinical applications. *Integr Med. Res.*, **6(1):** 12-18.10.

Khan, K.H. (2009). Roles of *Emblica officinalis* in medicine-a Review. *Bot. Res. Int.* **2(4):** 218-228.

Mittal, P.K., M. Anand, A.K. Madan, S. Yadav and J. Kumar (2014). Antioxidative capacity of vitamin E, vitamin C and their combination in cryopreserved Bhadavari bull semen,*Veterinary World.*, **7(12):** 1127-1131.

Osama, I.A. and K.H. Elias (2013). Effect of vitamins C or E supplementation to Tris diluent on the semen quality of Awassi rams preserved at 5°C, *Vet Res Forum*., **4(3):** 157-160.

Poonam, M. and L.M. Charu (2014). Comparative Analysis of Functional and Nutritive Values of Amla (Emblica officinalis) Fruit, Seed and Seed Coat Powder. *American Journal of Food Technology.*, **9:** 151-161.

Radwan Nadiia, L., R.A. Hassan, E.M. Qote and H.M. Fayek (2008). Effect of ntural antioxidant on oxidative stability of eggs and productive and Reproductive performance of laying hens. *Int. J. Poult. Sci.,* **7:** 134-150.

Rama, V.S., J.M. Gallardo Bolaños, L. González Fernández, J.A. Tapia, H. Rodríguez Martinez and F.J. Peña (2006). Identification of Sperm Subpopulations in Stallion Ejaculates: *Changes after Cryopreservation and Comparison with Traditional Statistics.*, **44:** 419-423.

Rizal, M., M.R. Teoliher, T.L. Yusuf, B. Purwantara and P. Situmorang (2003). Media Kedokteran Hewan 1979.

SAS (2012). SAS/STAT User's Guide for Personal Computers. Release 9.1 SAS Institute Inc., Cary, N.C., USA.

Schwenke, D.C. and S.R. Behr (1988). Vitamin E combined with selenium inhibits atherosclerosis in hypercholesterolemic rabbits independently of effects on plasma cholesterol concentrations. *Circ Res.*, **83:** 366-377.

Seddiki, Y., H. Moreira and F. Moreira (2017). Antioxidant Properties of Polyphenols and Their Potential Use in Improvement of Male Fertility: A Review, *Biomed. J. Sci. & Tech. Res.*, ISSN: 2574-1241.

Swanson, E.W. and H.J. Beardon (1951). An eosin-nigrosin stain differentiating live and dead bovine spermatozoa. *J. Anim. Sci.*, **10(4):** 981-987.

Walton, A. (1933). Technique of artificial insemination. *Mp. Bur. Anim. Genet. Iiius. Edinburgh*., 56.

World Health Organization (WHO) (2002). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva, World Health Organization (WHO).

Zribi, N., N. Feki Chakroun, F. Ben Abdallah, H. Elleuch and A. Sellami (2012). Effect of freezing-thawing process and quercetin on human sperm survival and DNA integrity. *Cryobiology.,* **65(3):** 326-331.