Use of Vitamin E to Improve Semen Quality of Awassi Rams During Chilled Preservation

Nameer Mahmood Albiaty Hazem Jawad Alobaidi Anmar Yousif Alnaeb
Abbas Fenjan Kareem Jasim Mohamad Jasim Majid Saadi Salman
Ali Moayd Al-Hakim
Ministry of Science and Technology / Agricultural and Biological Research Directorate - Animal and Fish Research Center
Baghdad - Iraq
E_mail: albiatyn99@yahoo.com

Abstract

The aim of this study was to investigate the possibility of improving chilled semen quality of Awassi rams by adding 0.0 mM vitamin E as control group and concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM as T1-T6 respectively. Treatments were preserved at 5˚C for 72 hrs. Results showed significant increase in motility when vitamin E was added. For dead sperm%, till 24 hrs of preservation, any of the experimental treatments could significantly differ from the control. T3-T6 showed lowest percentages of abnormal spermatozoa when preserved for 72 hrs. The proportion of defected acrosome was the highest in control group after 72 hrs. The study concluded that vitamin E has capability to improve biological characteristics of Awassi rams semen under chilled preservation; it can be added to the citrate egg yolk extender with recommended level of 3 mM.

Key Words: Awassi Ram Semen, Vitamin E and Chilled Preservation.

استخدام فيتامين E في تحسين نوعية السائل المنوي لكباش العواسي أثناء الحفظ بالتبريد

نمير محمود حلمي البياتي حازم جواد كاظم العبيدي انمار يوسف النائب عباس فنجان كريم
جاسم محمد جاسم ماجد سعدي سلمان علي مؤيد الحكيم
وزارة العلوم والتكنولوجيا / دائرة البحوث الزراعية – مركز الثروة الحيوانية والسمكية
بغداد– العراق

الخلاصة

هدفت الدراسة الحالية الى التعرف على امكانية تحسين نوعية السائل المنوي لكباش العواسي بإضافة فيتامين E الى المخفف بتركيز 0.0 ملي مول كمجموعة سيطرة و 0.5 ، 1.0 ، 1.5 ، 2.0 ، 2.5 و 3.0 ملي مول للمعاملات من T1 الى T6 على التوالي. بردت العينات بدرجة حرارة سليزية بمقدار 5 وحفظت لمدة 72 ساعة . اظهرت النتائج ان هناك زيادة معنوية في نسبة الحركة الامامية للنطف عند اضافة فيتامين E، وفي نسبة النطف الميتة فلغاية 24 ساعة خزن لم تختلف اي من المعاملات عن السيطرة. اظهرت المعاملات من الثالثة الى السادسة اقل نسب تشوهات للنطف عند الخزن 72 ساعة. كانت النسبة الأكبر لتشوهات الاكروسوم بعد 72 ساعة في مجموعة السيطرة . خلصت الدراسة الى ان لفيتامين E القدرة على تحسين الصفات البيولوجية للسائل المنوي لكباش العواسي عند التبريد، ويوصى باضافته بتركيز 3 ملي مول الى مخفف السترات – صفار البيض.

الكلمات المفتاحية : كباش العواسي ، السائل المنوي ، فيتامين E و الحفظ بالتبريد.

Introduction

In ram semen, the particular challenges of spermatozoa membrane are the excessively high proportion of poly unsaturated fatty acids (PUFA) / saturated fatty acids (SFA) and low proportion of cholesterol/ phospholipids (Holt, 2000), consequently, spermatozoa membrane will be vulnerable against peroxidative damages by reactive oxygen species (ROS) and subsequently causing lost of acrosome and membrane integrities (Alvarez and Storey, 1992). Beside, almost all semen characteristics could be adversely affected when ROS production was dominated on scavenging system (Beconi *et al.*, 1993; Anghel *et al.*, 2010). Vitamin E (α-tocopherol) is one of the main chain breaking and lipid soluble antioxidants found naturally in the mammal ejaculates, regulates ROS and protect spermatozoa from lipid peroxidation (Silva *et al*., 2013) , it can therefore scavenges all type of free radicals which may consisted in the preserved semen (Hamedani *et al.*, 2016).

Usually, artificial insemination centers presents balanced ration to their animals to keep good semen quality, but there are some limits, relatively high diet PUFA/ SFA ratio has been shown to increase the demands of vitamin E to gets rid of the impact of ROS production (Jager, 1972). In addition, animal feeding regimens in some countries have a shortages in feedstuff supply which caused deficient in vitamin E, or the animal being under sever climate, or there are a genetically superior ram but has poor semen quality. Therefore, the aim of this study was to investigate the possibility of improving chilled semen quality of Awassi rams by supplements of vitamin E into the citrate- egg yolk extender.

Materials and Methods

The study was conducted in the Ruminant Department, Animal and Fish Research Center on three Awassi rams aged 2 – 3 years and body condition score 2.0 – 2.5. Semen was collected using electro ejaculator (Electro jac - 5), immediately transferred to the laboratory and incubated in water bath at 37˚C for raw biological evaluation. Samples which did not met the following limits were ignored; volume, 0.5 – 2.0 ml; mass motility, 3 – 5; progressive motility, < 75%; dead sperm, >10% and abnormal sperm, >20%. Semen of the three rams were pooled to get rid of ram effect, pooled semen then was subdivided into seven equal aliquots and were diluted by citrate – egg yolk extender to get final concentration of 5×10^6 sperm/ ml. The standard citrate – egg yolk extender was prepared by mixing 2.37 gm Trisodium citrate, 0.5 gm Glucose, 100 ml distilled water, and 20% (v/v) egg yolk. Treatment with 0.0 mM vitamin E (α – tocopherol) were considered as control (C), other experimental concentrations were 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM of vitamin E supplemented as T1 to T6 respectively, all tubes were heated to 50˚C to homogenize the contents then let to cool to room temperature, penicillin (100000 IU) and streptomycin (100 mg) were then added. After dilution, semen tubes were gradually cooled from 32 to 5˚C within two hours using programmable incubator (Sanyo, MIR- 253) then kept in 5˚C for preservation for 72 hours in which evaluation of the treatments were applied after dilution, cooling, 24, 48 and 72 hrs of preservation. Semen

evaluation was conducted by conventional procedures. Mass motility was estimated at 1 – 5 scale, X_{40} magnification (Avdi *et al*., 2004). Progressive motility was estimated as forward sperm movement % under X_{400} magnification according to (Soltanpour and Moghddam, 2014), sperm concentration was determined by special spectrophotometer (Densimeter – 591). Dead and abnormal sperm percentages have been viewed under X_{400} and X_{1000} magnification (oil immersion) respectively of eosin – nigrosin stains slides (Salamon and Maxwell, 1995). Acrosome defects (%) was determined using eosin – fast green stains procedure (Wells and Awa, 1970) and 200 sperm in different microscopic field (X_{1000} oil immersion magnification) have been seen. This trail was replicated once a week for six times. Statistical analysis were performed at general linear model of SPSS program (SPSS soft ware, 2001), taking into account that $p \leq 0.05$ is the maximum level for the significant differentiation of the means.

Results and Discussion

Table (1) revealed the effects of adding vitamin E to the extender on sperm motility, there were no significant differences among treatments in early stages of semen preparation, while significant increase ($p \leq 0.05$) have been shown later as T6 (3 mM vitamin E) has the highest (51.67%; $p \leq 0.05$) and C (0.0% vitamin E) has the lowest motility (37.5%; $p \leq 0.05$) after 72 hrs of preservation. This increase of motility may attribute to the efficiency of vitamin E in citrate - egg yolk extender as antioxidant which confirmed by the finding of Asadpour *et al*., (2011) and Soltanpour *et al*., (2014) on bulls and rams semen respectively. Prolonged preservation period to 72 hrs led vitamin E to exhibit their capability of scavenging ROS from the extender and make the difference with control. In contrast, Aguero *et al*., (1995) reported that supplementing extender with vitamin E improved maintaining good motile and viable spermatozoa of stallion during 24 hrs in cooled preservation.

Table (2) represented dead sperm percentages in different levels of vitamin E supplementation. Till 24 hrs of chilled preservation at 5˚C, any of the experimental treatments could significantly differed from the control, but after 72 hrs, T6 recorded lowest ($p \leq 0.05$) percentage (22.27%) compared with control and other treatments. The differences have been appeared first time after 48 hrs with lowest percentage of T6. Any increase in vitamin E caused a decrease in dead sperm percentages. Regarding the viability of spermatozoa, it seems that 3 mM of vitamin E was suitable to Awassi rams semen preservation and the adverse effects of PUFA peroxidation, which affected spermatozoa viability by free radicals production could be controlled under the external supplements of vitamin E. Hamedani *et al*., (2016) agreed that the decrease of live spermatozoa in control group can be attributed to the peroxidation of PUFA and phospholipids to generate peroxyl, superoxide and hydroxyl radicals.

Table (1) Effect of Vitamin E Addition to the Extender on Sperm Motility (%) of Awassi Ram Semen in Preparation Stages and Preservation at 5˚C (mean ± SEM).

Stage / Vitamin E	Dilution	Cooling	24 hrs Preservation	48 hrs Preservation	72 hrs Preservation
Control	78.33 a ± 2.17	66.67 a ± 1.98	63.33 ab ± 2.34	54.17 b ± 1.81	37.50 c ± 3.80
T1	78.33 a ± 1.76	65.83 a ± 2.93	64.17 ab ± 2.82	56.67 b ± 2.82	45.33 ab ± 2.86
T2	80.83 a ± 1.82	70.00 a ± 1.87	62.50 ab ± 1.94	56.67 b ± 1.41	42.50 bc ± 2.62
T3	80.00 a ± 2.95	67.50 a ± 1.29	65.83 ab ± 3.15	60.00 ab ± 3.73	42.50 bc ± 2.12
T4	79.16 a ± 3.12	70.00 a ± 3.14	58.33 b ± 1.04	56.67 b ± 4.91	45.83 ab ± 1.83
T5	80.83 a ± 2.79	65.83 a ± 1.91	69.17 a ± 4.89	65.83 a ± 3.71	46.67 ab ± 3.64
T6	80.00 a ± 2.16	68.33 a ± 2.24	69.17 a ± 4.54	66.67 a ± 1.98	51.67 a ± 2.53

Control=0.0mM, T1=0.5mM, T2=1mM, T3=1.5mM, T4=2mM, T5=2.5mM, T6=3mM
Different superscripts within column are significantly different (P ≤ 0.05).

Table (2) Effect of Vitamin E Addition to the Extender on Dead Sperm (%) of Awassi Ram Semen in Preparation Stages and Preservation at 5˚C (Mean ± SEM).

Stage / Vitamin E	Dilution	Cooling	24 hrs Preservation	48 hrs Preservation	72 hrs Preservation
Control	16.40 a ± 1.98	24.75 a ± 1.52	29.00 a ± 1.34	37.06 a ± 2.14	38.67 a ± 2.92
T1	13.56 a ± 1.54	22.10 a ± 1.62	25.73 a ± 1.98	26.59 bcd ± 3.98	34.52 ab ± 2.76
T2	13.45 a ± 1.51	20.90 a ± 1.91	27.24 a ± 2.25	27.21 bc ± 1.84	32.18 ab ± 1.06
T3	13.20 a ± 1.87	18.45 a ± 0.91	23.36 a ± 2.93	29.93 b ± 1.34	32.13 ab ± 3.85
T4	11.22 a ±2.03	20.78 a ± 0.96	25.19 a ± 1.19	22.97 cd ± 1.84	30.12 b ± 3.73
T5	10.15 a ± 1.51	20.54 a ± 1.38	27.63 a ± 1.61	21.51 cd ± 2.96	30.16 b ± 2.21
T6	12.57 a ± 1.76	18.21 a ± 1.51	23.52 a ± 2.13	19.74 d ± 3.83	22.27 c ± 2.15

Control=0.0mM, T1=0.5mM, T2=1mM, T3=1.5mM, T4=2mM, T5=2.5mM, T6=3mM
Different Superscripts within Column are Significantly Different (P ≤ 0.05).

Also, the unique chemical structure of vitamin E (hydrophilic *vs.* hydrophobic lateral sides) makes it more potential against lipid peroxidation (Salamon and Maxwell, 1995; Negis *et al*., 2007). Abnormal sperm percentages have been shown in Table (3), there were no significant differences in all treatments after dilution, while increasing abnormalities takes place in control group to be significant (p≤ 0.05) at 72 hrs of storage, it is relevant to mention that extender free of vitamin E cause increase of abnormal sperm proportion. Pour *et al*., (2013) reported that vitamin E was able to protect spermatozoa during storage at 5˚C and freezing condition. T3, T4, T5, and T6 showed lower (p≤ 0.05) percentages of abnormal spermatozoa for 72 hrs under chilled preservation at 5˚C. High dose

of vitamin E in this study may be benefit to improve preservation circumstances, so, it could be useful method to save poor quality semen of genetically superior males. Higher supplementation of α- tocopherol (2-3 mM) into the extender have been recommended by Hamedani *et al*.,(2016) due to the best phenotypic normality of spermatozoa, in contrast, Asadpour *et al*.,(2011) concluded that lower concentration of vitamin E (0.1 and 0.2 mM) was very efficient antioxidants for bull spermatozoa.

Table (4) showed the proportion of acrosome defects in different supplementation of vitamin E. Directly after dilution, the differences were not significant and means ranged between (6.49-10.83 %), after the samples reached their storage temperature (5˚C) significant differences (p≤ 0.05) have been shown. The proportion of defected acrosome was the highest (p≤ 0.05) in control group after 72 hrs (20.73%) while the lowest (8.83%) was in T6, all of the experimental treatments showed significant superiority (p≤ 0.05) in comparison with control. These facts indicated the potent of different supplementation of vitamin E into the citrate egg yolk extender, due to the particular properties of α- tocopherol as lipo - soluble antioxidant molecules dispersed into the plasma membranes, ROS production from PUFA peroxidation will be blocked. Alvarez and Storey (1992) was in line that peroxidative damage caused by ROS could lose acrosome integrity, the efficient mechanism of vitamin E in membranes (Rezk *et al*., 2004) makes it important additive to the ram

Table (3) Effect of Vitamin E Addition to the Extender on Abnormal Sperm (%) of Awassi Ram Semen in Preparation Stages and Preservation at 5˚C (Mean ± SEM).

Stage / Vitamin E	Dilution	Cooling	24 hrs Preservation	48 hrs Preservation	72 hrs Preservation
Control	10.75 a ± 0.86	14.62 a ± 0.69	14.43 a ± 1.98	17.94 a ± 1.71	20.51 a ± 1.19
T1	9.08 a ± 0.16	10.13 ab ± 0.54	13.62 ab ± 1.52	12.53 ab ± 1.96	13.98 b ± 2.94
T2	9.73 a ± 0.94	7.63 b ± 0.81	13.94 ab ± 0.63	10.79 b ± 1.52	12.63 b ± 1.90
T3	9.54 a ± 0.19	10.71 ab ± 0.93	10.73 ab ± 0.86	11.81 b ± 1.82	11.04 bc ± 2.82
T4	7.93 a ± 0.94	10.76 ab ± 1.38	12.24 ab ± 0.19	10.81 b ± 1.93	11.94 bc ± 2.04
T5	6.41 a ± 1.02	8.83 ab ± 1.76	9.75 bc ± 1.98	9.06 b ± 1.91	11.84 bc ± 2.62
T6	6.72 a ± 0.47	9.84 ab ± 1.49	6.95 c ± 1.48	8.81 b ± 1.82	8.96 c ± 2.29

Control=0.0mM, T1=0.5mM, T2=1mM, T3=1.5mM, T4=2mM, T5=2.5mM, T6=3mM
Different Superscripts within Column are Significantly Different (P ≤ 0.05).

extenders. However, as vitamin E was added to the extender, early significant improvement (Cooling Stage) appeared on the studied characteristic have been shown in the percentages of abnormal and acrosome defect which clearly indicate that vitamin E sooner interacts with the spermatozoa cytoplasmic and acrosomal membranes to repair it and prevent the deterioration effects of free radicals (Anghel *et al*., 2010).

Figure (1) illustrates effects of dilution, cooling and preservation periods (independent of treatments) on some semen characteristics of Awassi rams. All studied characteristics have been significantly deteriorated ($p \leq 0.05$), progressive motility was steadily declined in all stages while dead, abnormal and acrosome defect percentages were increased, it is usual phenomenon due to the biological system of live cells, Soltanpour *et al*., (2014) and albiaty *et al*., (2016) confirmed these facts. Indeed, determining the time in which significant deterioration of semen characteristics take place is of great important to select the stage having suitable semen quality for subsequent artificial insemination works. In conclusion, vitamin E is a unique lipid soluble antioxidant, it could be added to the citrate egg yolk extender due to its capability to improve biological characteristics of Awassi rams semen, with recommended level of 3 mM.

Table (4) Effect of Vitamin E Addition to the Extender on Acrosome Defect (%) of Awassi Ram Semen in Preparation Stages and Preservation at 5°C (Mean ± SEM).

Stage / Vitamin E	Dilution	Cooling	24 hrs Preservation	48 hrs Preservation	72 hrs Preservation
Control	10.83 a ± 0.81	14.91 a ± 1.18	14.83 a ± 1.81	17.82 a ± 1.81	20.73 a ± 1.01
T1	9.05 a ± 1.91	10.71 ab ± 0.91	13.81 ab ± 1.75	12.23 ab ± 1.15	13.31 b ± 0.84
T2	9.94 a ± 0.79	7.92 b ± 0.53	13.69 ab ± 0.98	10.58 b ± 0.81	12.24 b ± 0.19
T3	9.92 a ± 1.81	10.45 ab ± 0.61	10.65 ab ± 1.17	11.04 b ± 0.87	11.04 bc ± 0.89
T4	7.24 a ± 0.71	10.52 ab ± 0.16	12.72 ab ± 1.42	10.92 b ± 0.41	11.83 bc ± 1.01
T5	6.74 a ± 0.81	8.72 ab ± 1.06	9.82 bc ± 1.49	9.94 b ± 1.18	11.72 bc ± 0.81
T6	6.49 a ± 1.91	9.05 ab ± 1.08	6.54 c ± 0.92	8.91 b ± 1.31	8.83 c ± 0.88

Control=0.0mM, T1=0.5mM, T2=1mM, T3=1.5mM, T4=2mM, T5=2.5mM, T6=3mM
Different Superscripts within Column are Significantly Different (P ≤ 0.05).

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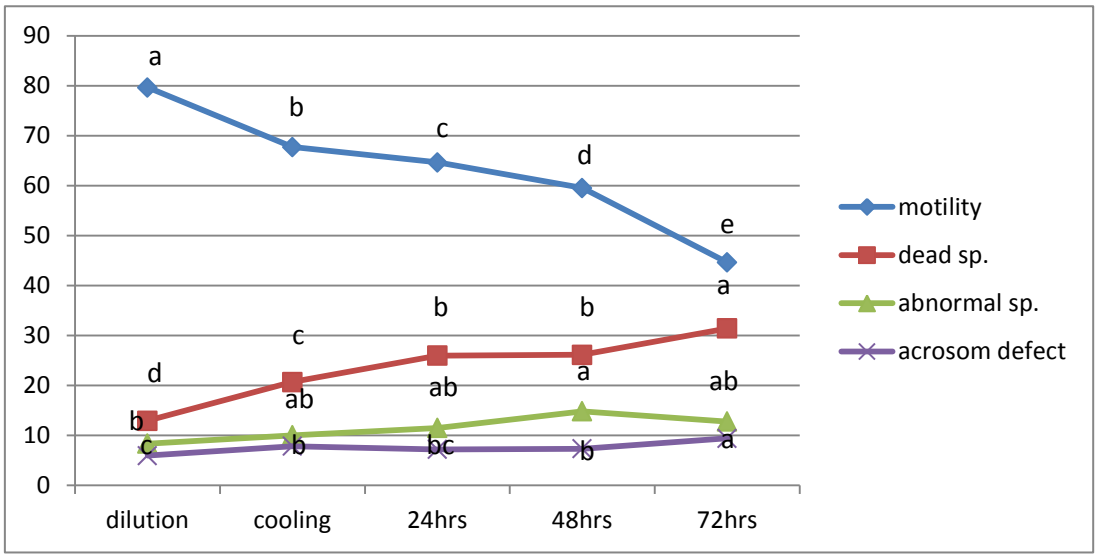

Fig. (1) Effect of Processing and Preservation Stages (Independent of Treatments) on some Semen Characteristics of Awassi Rams. Different Superscripts within the Same Characteristics were Significantly Differed (P ≤ 0.05).

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