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EFFECT OF ADDING α-LIPOIC ACID ON SOME POST-CRYOPRESERVED SEMEN CHARACTERISTICS OF HOLSTEIN BULLS

Sajeda Mahdi Eidan[1], Ali J. Al-Nuaimi[2], Omar Amer Abd Sultan[3], Faris Faisal Ibrahim[4] and Talal Anwer Abdulkareem[1] and Wafaa Edam Lateef [4]

[1]Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.
[2] College of Veterinary Medicine, University of Kerbala, Iraq.
[3] Directorate of Combat Desertification, Ministry of Agriculture, Iraq.
[4]Department of Artificial Insemination, Directorate of Animal Resource, Ministry of Agriculture, Iraq.

Abstract

This study was undertaken to explore the adding effect of α-lipoic acid to Tris extender on some post-cooling and post-cryopreserved semen characteristics of Holstein bulls for different preservation periods (cooling at 5ºC, 48 h, 1,2 and 3 months post-cryopreservation; PC). Seven Holstein bulls of 3-4 years old were used in this experiment. Semen was collected via artificial vagina in one ejaculate per bull per week for the 7 weeks experimental period. Pooled semen was equally divided into three groups using Tris extender. Two levels of α-lipoic acid were added to Tris extender being 0.5 (L_2) and 1(L_3) mM and comparisons in response were made with the control group (Tris extender, L_1). Total motile spermatozoa (TMS), total normal morphology of sperms (TNMS), total acrosomal integrity of sperms (TAIS), total plasma membrane integrity of sperms (TPMIS), total osmotic shock of sperms (TOSS) and total function sperm fraction (TFSF) were estimated per straw ($\times 10^6$) for each group and preservation period. Excluding data of TOSS, all other sperm characteristics number per straw were greater for L_2 and L_3 groups as compared with the L_1 group. In conclusion, adding of 0.5 and 1 mM of α-lipoic acid to Tris extender resulted in increasing PC sperm characteristics number per straw for Holstein bulls.

Keywords: α-lipoic acid, Tris, semen, post-cryopreservation, Holstein bulls.

Introduction

The improvement in quality of semen is an important aspect for maximum utilization of genetically superior sub-fertile sires, for the reason of age and \ or non-specific factors like heat, transport and vaccination stresses during their dynamic life show a low grade semen quality than previously. The reduction and, loss in viability and fertility can be attributed to many changes take place in the sperm cells during cryopreservation (Rao *et al*., 2013). Cryopreservation (which associated with oxidative stress) affects the lipid architecture of plasma membrane (Mazur *et al.,* 2000 and Kumaresan *et al.*, 2006) and metabolism due to high amount of polyunsaturated fatty acids (PUFAs) with significantly less cytoplasmic components containing antioxidants (Andrabi, 2009).

The process of the bulls semen cryopreservation in often leads to low levels of antioxidants such as GSH , Glutathione , and Superoxide dismutase (SOD) in sperm cells (Sarioskan *et al.,* 2009, Eidan *et al.,* 2015 and Abdulkareem *et al.,* 2018a,b,c) .The addition of some industrial enzymes antioxidants such as GSH and catalase (Eidan, 2016) and non – enzymatic industries such as vitamin A , E and C (Al-Zaidi, 2014, Eidan *et al.,* 2015b, Abdulkareem and Al-Zaidi, 2018a,b) amino acids (Mohammad *et al.,* 2014, Abdulkareem *et al.,* 2016), manganese (Eidan *et al.,* 2015a) and coenzyme Q10 (Sultan, 2015) and their various combinations to Tris extender (Abdulkareem *et al.,* 2017a ,b) of bulls Holstein in Iraq have given good results in improving the quality of semen post- cryopreservation (PC).

Alpha-lipoic acid or 1, 3-dithiolane-3-pentanoic acid, is a naturally occurring dithiol compound synthesized enzymatically in the mitochondrion from octanoic acid, and can be found naturally in mitochondria (Berg *et al.,* 2007). This compound is responsible as a coenzyme for pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase. Hence, it is essential for energy production (Long *et al.,* 2009). Moreover, α-lipoic acid or its reduced form, dihydrolipoic acid (DHLA) can improved the harmful effect of oxidative stress both in aqueous and membrane phases. Also, α-lipoic acid and DHLA appear to have the ability to regenerate vitamin C and thus, indirectly recycles vitamin E (Packer *et al.,* 2001).

Ibrahim *et al.* (2008) found that adding 0.02 mmol\ml of α-lipoic acid to Tris extender of Boer buck's semen improved post-cryopreserved (PC) sperm's cell individual motility and reduced sperm's DNA damage. In Iraq, Sultan (2015) and Eidan *et al* (2017) figure out that adding of 0.5 mM of α- lipoic acid to Tris extender enhanced PC sperm's cell individual motility, live sperm, sperms plasma membrane and acrosome integrity as well as sperm's freezability percentages of Holstein bulls. However, the influence of adding of α-lipoic acid to Tris extender on PC sperm characteristics number per straw for Holstein bulls did not previously determined. Therefore, this study was carried out.

Materials and Methods

Seven Holstein bulls, 3.5- 4.5 years old and 550-770 kg live body weight, were trained for semen collection using artificial vagina. The experimental bulls have good health and free of disease, being under the veterinarian supervision permanently. Animals fed 4 kg concentrate ration/ bull /day composed of 33% wheat bran, 20% soybean, 35% barley, 10% yellow corn, 0.5% salt 0.5% limestone, and 1% vitamins and minerals. Total crude protein and energy contents were 18% and 3323 kcal / kg respectively. Green fodder (51-61 kg / bull / day) alfalfa hay (8-9 kg/bull /day) were also introduced. Pooled semen was taken from each bull and combining together to remove the individual variations

among bulls. The extender was prepared according to the method of Salamon and Maxwell (2000). Semen was divided equally to three groups. First group was considered as a control group (A_1) diluted with Tris only. α- lipoic acid was added to A_2 (0.5 mM) and A_3 (1.0 mM) groups respectively. Semen evaluation was done on each treatment following cooling (5° C) and 72 hours, 1st, 2nd and 3rd PC periods. Total motile spermatozoa (TMS), total normal morphology of sperms (TNMS), total acrosomal integrity of sperms (TAIS), total plasma membrane integrity of sperms (TPMIS), total osmotic shock of sperms (TOSS) and total function sperm fraction (TFSF) were estimated per straw ($\times 10^6$) for each group and preservation period according to Correa and Zavos (1994) and Eidan (2016).

The statistical computations were performed using SAS program (2012) based on completely randomized design (CRD) to study the effect of different factors on the studied characteristics. Means with significant differences were compared using Duncan multiple range test (1955).

Results and Discussion

Total motile spermatozoa (TMS)

Total motile spermatozoa ($\times 10^6$) in each straw was superior ($p \leq 0.0001$-$p \leq 0.002$) in A_2 (8.42±0.29-11.28±0.42) and A_3 (7.42±0.52 - 10.28±0.52) groups in comparison with A_1 group (5.42±0.29-8.42±0.52) following different preservation periods (Table1).On the other hand, non-significant differences were found between A_2 and A_3 at the whole PC periods, except for 2nd month PC which revealed clear differences ($p \leq 0.0001$) between them (Table 1).

Within A_1 and A_2 groups, highly significant differences ($p \leq 0.0001$) in TMS were noticed at cooling as compared with the remaining PC periods (Table 1). Meanwhile, non-significant differences within all groups were noticed in TMS ($\times 10^6$) at 1st, 2nd and 3rd month PC (Table 1). The TMS increased ($p \leq 0.003$) at cooling period as compared with 1st, 2nd and 3rd month PC periods within A_3 group (Table 1).

Total normal morphology of sperms (TNMS)

The A_2 (16.86±0.11-16.97±0.10) and A_3 (16.31±0.13-16.55±0.12) groups exhibited an obvious differences ($p \leq 0.0001$-$p \leq 0.0008$) in TNMS ($\times 10^6$) in each straw as compared with A_1 group (15.72±0.09-15.98±0.10) at cooling, 48hr, 1st and 2nd, but not 3rd month PC periods. (Table 2).The A_2 group exhibited higher ($P \leq 0.0001$) TNMS ($\times 10^6$) values as compared with C3 group at cooling (16.89±0.08 vs. 16.31±0.13) and 48 hr. (16.97±0.10 vs.16.55±0.12) periods (Table 2). Non-significant differences were noticed among the whole preservation periods in TNMS ($\times 10^6$) within each group (Table 2).

Total acrosomal integrity of sperms (TAIS)

The A_2 and A_3 groups exhibited the greatest ($P \leq 0.0001$-$P \leq 0.0005$) TAIS values as compared with A_1 group at all PC periods (Table 3). The current results showed that A_2 group had significantly increased ($P \leq 0.0001$) TAIS ($\times 10^6$) as compared with A_3 group at 1st, 2nd and 3rd month PC (Table 3). Meanwhile, the differences between A_2 and A_3 groups at cooling and 48 hr PC periods lacked significance (Table 3).

Within A_1 group, the TAIS were greater ($P \leq 0.001$) at cooling in comparison with1st, 2nd and 3rd month PC (Table 3). Moreover, Non- significant differences were noticed among 1st, 2nd and 3rd month PC within the relevant group (Table 3). Within A_2 and A_3 groups, the TAIS were greater ($P \leq 0.007$, $P \leq 0.03$) at cooling in comparison with 2nd and 3rd month PC (Table 44). The differences observed among the PC periods within these two groups lacked significance (Table 3).

Total plasma membrane integrity of sperms (TPMIS)

The TPMI ($\times 10^6$) was significantly greater ($P \leq 0.0001$) in A_2 (15.36±0.09) in comparison with both A_3 (14.31±0.18) and A_1 (12.85±0.32) groups at cooling period (Table 4). The A_3 group exhibited higher value in TPMIS as compared with A_1group at cooling period (Table 3). An obvious ($P \leq 0.0009$-$P \leq 0.001$) effect of two α- lipoic acid levels was noticed on TPMIS at 48 hr, 1st and 2nd months PC as compared with A_1 group (Table 4). The A_2 group recorded greater TPMIS value (13.85±0.31) in comparison with A_1 group (12.01±0.39) at 3rd month PC (Table 4).

In A_1 group, non-significant differences in TPMIS ($\times 10^6$) were noticed at different preservation periods (Table 4). Cooling period exhibited greater ($p \leq 0.002$) TPMIS value as compared with 1st, 2nd and 3rd months PC period within the A_2 group (Table 4). The cooling and 48 hr periods revealed higher differences ($P \leq 0.01$) in TPMIS as compared with 3rd month PC in A_3 group (Table 4). Differences in TPMI in A_2 and A_3 groups did not register among the most PC periods (Table 4).

Total osmotic shock of sperms (TOSS)

The total osmotic shock of sperms ($\times 10^6$) decreased ($P \leq 0.0001$- $P \leq 0.0007$) in A_2 group as compared with both A_3 and A_1 groups at all experimental periods (Table 5). Moreover, the A_3 group exhibited lesser ($P \leq 0.0001$-$P \leq 0.0007$) TOSS value in comparison with A_1 at cooling, 48 hr and 1st month PC periods (Table 5). Non-significant differences were noticed among the overall experimental periods within each group (Table 5).

Total function sperm fraction (TFSF)

The A_2 exhibited higher ($p \leq 0.0001$) TFSF ($\times 10^6$) values as compared with both C_1 and C_3 groups during all the experiment periods (Table 6). At similar period, significant differences ($P \leq 0.0001$) were found between A_3 and A_1 at all the experimental periods (Table 6).

Significant differences ($P \leq 0.0002$, $P \leq 0.0001$) were noticed in TFSF values between cooling and the remaining PC periods in both A_1 and A_2 groups (Table 6). In A_3 group, TFSF was greater ($P \leq 0.0009$) at cooling (6.01±0.35) as compared with 1st (4.41±0.35), 2nd (4.14±0.31) and 3rd (3.96±0.30) months PC (Table 6). Moreover, 48 hr period were superior ($P \leq 0.0001$, $P \leq 0.0009$) in TFSF values as compared with those at 2nd and 3rd months PC in A_2 and A_3 groups (Table 6).Within each group, Non-significant differences were noticed among the most PC periods (Table 6).

Table 1 : The Effect of adding α-lipoic acid to Tris extender on the total motile spermatozoa (TMS; $\times 10^6$) at cooling and different post-cryopreservation (PC) periods in Holstein bulls.

Period / Group	Cooling (5˚ C)	48 hr PC	First month PC	Second month PC	Third month PC	Level of significance
A_1	8.42±0.52 B a	6.85±0.40 B b	5.71±0.35 B c	5.57±0.29 C c	5.42±0.29 B c	P≤0.0001
A_2	11.28±0.42 A a	9.85±0.26 A b	9.00±0.30 A bc	8.71±0.35 A c	8.42±0.29 A c	P≤0.0001
A_3	10.28±0.52A a	8.85±0.55 A ab	7.71±0.60 A b	7.42±0.52 B b	7.42±0.52 A b	P≤0.003
Level of significance	P≤0.002	P≤0.0003	P≤0.0002	P≤0.0001	P≤0.0001	

Means with capital superscripts within each column indicated comparison among groups and small superscripts within each row indicate comparison among periods within each group.
A_1: Tris extender (Control group).
A_2: Tris extender + 0.5 mM α- lipoic acid.
A_3: Tris extender + 1.0 mM α- lipoic acid.

Table 2 : The Effect of adding α-lipoic acid to Tris extender on the total normal morphology (TNMS)($\times 10^6$) of sperms at cooling and different post-cryopreservation (PC) periods in Holstein bulls.

Period / Group	Cooling (5˚ C)	48 hr PC	First month PC	Second month PC	Third month PC	Level of significance
A_1	15.72±0.09C a	15.86±0.08 C a	15.94±0.09 B a	15.98±0.10 B a	15.96±0.18 A a	N.S
A_2	16.89±0.08A a	16.97±0.10 A a	16.87±0.11 A a	16.86±0.11 A a	16.38±0.39 A a	N.S
A_3	16.31±0.13B a	16.55±0.12 B a	16.48±0.17 A a	16.47±0.17 A a	16.4±0.14 A a	N.S
Level of significance	P≤0.0001	P≤0.0001	P≤0.0004	P≤0.0008	N.S	

Means with capital superscripts within each column indicate comparison among groups and small superscripts within each row indicate comparison among periods within each group.
NS: No significant.
A_1: Tris extender (Control group).
A_2: Tris extender + 0.5 mM α- lipoic acid.
A_3: Tris extender + 1.0 mM α- lipoic acid.

Table 3 : The Effect of adding α-lipoic acid to Tris extender on the total acrosomal integrity (TAIS$\times 10^6$) of sperms at cooling and different post-cryopreservation (PC) periods in Holstein bulls.

Period / Group	Cooling (5˚ C)	48 hr PC	First month PC	Second month PC	Third month PC	Level of significance
A_1	13.60±0.25 B a	13.35±0.33 B ab	12.78±0.22 C bc	12.58±0.20 C c	12.11±0.15 C c	P≤0.001
A_2	15.89±0.34A a	15.44±0.24 A ab	15.33±0.18 A ab	14.82±0.17 A bc	14.55±0.27 A c	P≤0.007
A_3	14.97±0.32A a	14.66±0.32 A ab	14.04±0.26 B abc	13.83±0.36 B bc	13.51±0.41 B c	P≤0.03
Level of significance	P≤0.0002	P≤0.0005	P≤0.0001	P≤0.0001	P≤0.0001	

Means with capital superscripts within each column indicate comparison among treatments and small superscripts within each row indicate comparison among periods within each treatment.
C1: Tris extender (Control group).
C2: Tris extender + 0.5 mM α- lipoic acid.
C3: Tris extender + 1.0 mM α- lipoic acid.

Table 4 : The Effect of adding α-lipoic acid to Tris extender on the total plasma membrane integrity of sperms (TPMIS×10^6) of sperms at cooling and different post-cryopreservation (PC) periods in Holstein bulls.

Period / Group	Cooling (5˚ C)	48 hr PC	First month PC	Second month PC	Third month PC	Level of significance
A_1	12.85±0.32C a	12.7±0.15 B a	12.76±0.28 B a	12.15±0.39 B a	12.01±0.39 B a	N.S
A_2	15.36±0.09A a	14.90±0.24 A ab	14.49±0.24 A bc	14.28±0.27 A bc	13.85±0.31 A c	P≤0.002
A_3	14.31±0.18B a	14.28±0.30 A a	13.86±0.30 A ab	13.54±0.30 A ab	13.0±0.31 AB b	P≤0.01
Level of significance	P≤0.0001	P≤0.0001	P≤0.001	P≤0.0009	P≤0.005	

Means with capital superscripts within each column indicate comparison among groups and small superscripts within each row indicate comparison among periods within each group.
NS: No significant.
A_1: Tris extender (Control group).
A_2: Tris extender + 0.5 mM α- lipoic acid.
A_3: Tris extender + 1.0 mM α- lipoic acid.

Table 5 : The Effect of adding α-lipoic acid to Tris extender on the total osmotic shock (TOSS×10^6) at cooling and different post-cryopreservation (PC) periods in Holstein bulls.

Period / Group	Cooling (5˚ C)	48 hr PC	First month PC	Second month PC	Third month PC	Level of significance
A_1	1.70±0.08 A a	1.56±0.02 A a	1.59±0.03 A a	1.60±0.04 A a	1.55±0.05 A a	N.S
A_2	1.26±0.05 C a	1.20±0.06 C a	1.18±0.05 C a	1.14±0.04 B a	1.15±0.05 B a	N.S
A_3	1.50±0.05 B a	1.40±0.05 B a	1.43±0.05 B a	1.48±0.04 A a	1.47±0.04 A a	N.S
Level of significance	P≤0.0007	P≤0.0002	P≤0.0001	P≤0.0001	P≤0.0002	

Means with capital superscripts within each column indicate comparison among groups and small superscripts within each row indicate comparison among periods within each group.
NS: No significant.
A_1: Tris extender (Control group).
A_2: Tris extender + 0.5 mM α- lipoic acid.
A_3: Tris extender + 1.0 mM α- lipoic acid.

Table 6 : The Effect of adding α-lipoic acid to Tris extender on the total function sperm fraction (TFSF×10^6) at cooling and different post-cryopreservation (PC) periods in Holstein bulls.

Period / Group	Cooling (5˚ C)	48 hr PC	First month PC	Second month PC	Third month PC	Level of significance
A_1	4.29±0.36 C a	3.45±0.22 C b	2.91±0.19 C bc	2.72±0.20 C bc	2.61±0.19 C c	p≤0.0002
A_2	7.32±0.29 A a	6.23±0.17 A b	5.49±0.17 A c	5.24±0.20 A cd	4.78±0.24 A d	p≤0.0001
A_3	6.01±0.35 B a	5.24±0.35 B ab	4.41±0.35 B bc	4.14±0.31 B c	3.96±0.30 B c	p≤0.0009
Level of significance	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001	

Means with capital superscripts within each column indicate comparison among groups and small superscripts within each row indicate comparison among periods within each group.
A_1: Tris extender (Control group).
A_2: Tris extender + 0.5 mM α- lipoic acid.
A_3: Tris extender + 1.0 mM α- lipoic acid.

This is the first study described the influence of adding high α-lipoic acid concentrations (0.5 and 1 mM) to Tris extender on PC semen attribute number per straw in Holstein bulls. Adding of 0.5 and 1 mM of α-lipoic acid had an overwhelming effect in improving all PC sperm characteristics number per straw for Holstein bulls. The current results were also agreed with Gohar *et al* (2014) who observed that adding of 0.5 and 1mM α-lipoic acid to Tris extender improved PC sperm's cell individual motility, plasma membrane and acrosome integrity percentages of buffalo semen. These results were also consistent with those reported by Sultan (2015) who noticed that adding of 0.5 and 1 mM of α-lipoic acid to Tris extender improved most PC semen characteristics of Holstein bulls.

The enhancement of the current semen characteristics may be due a potent biological antioxidant and a detoxification role of α-lipoic acid (Shay *et al.,* 2009). The α-lipoic acids have assisted in the metabolism of oxidative decarboxylation by acting as a co-enzyme (Haenen and Bast, 1991). The increase in oxidative decarboxylation would increase cytochrome C concentration and, thus, directly increase the mitochondria's membrane potential, improving regulation of mitochondria function and its biogenesis (Gopalakrishnan and Scarpulla, 1994). Moreover, α-lipoic has also been reported to assist the mitochondria's citric cycle. This in turn, will increase the level of reduced glutathione, ATP, TCA cycle enzyme and electron transport chain complex activities (Palaniappan and Dai, 2007). The α-lipoic acid regulation of metabolism, increased availability of mitochondrial co-enzymes and improvement of protection of free radicals are thought to eventually lead to a reduced incidence of mitochondria dysfunction, thus, ensuring sufficient ATP for sperm movement (Moreira *et al.,* 2007). The oxidized and reduced α-lipoic acid forms create a potent redox couple that has a standard reduction potential.

In conclusion, adding of α-lipoic acid (0.5 and 1 mM) to Tris extender inhibits LPO, and thus, increasing the membrane integrity and the whole semen characteristic at cooling and PC periods. Greater fertility and pregnancy rates are expected to achieve in cows artificially-inseminated with straws contained α-lipoic acid. Due to these overwhelming antioxidant properties of α-lipoic acid, through improving the above-mentioned semen characteristics, the current study is strongly recommended to apply at the artificial insemination centers in Iraq and worldwide.

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