Effect of adding Manganese chloride and Co-enzymes (α-lipoic acid and Q10) on post-cryopreservation semen quality characteristics of Holstein bulls

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Summary

This study was undertaken to explore the adding effect of manganese chloride ($MnCl_2$), co-enzyme Q10 (Co-Q10) as well as α-lipoic acid to Tris extender on Freeze ability of Holstein bulls' semen. This study was carried out at the department of artificial insemination at the Directorate of Animal Resource, Ministry of Agriculture in Abu-Ghraib, Baghdad for the duration from October\ 2013 to Jun\ 2014, including three experiments. Seven Holstein bulls of 3.5-4 years old were used in this study. Semen was collected via artificial vagina by one ejaculate/ bull/ week. The assessments were conducted for fresh semen, which was later pooled, equally divided for various treatments within each experiment, using Tris extender. In the first experiment, pooled semen was divided into three groups. First group was diluted with Tris only. Manganese chloride was added to Tris extender (0.7 mM) in the 2nd group while in 3rd group (0.9 mM) of the Manganese chloride was used. In the second experiment, semen was divided equally into three groups. The first group was considered as a control group diluted with Tris only. Co-enzyme Q10 was added to 2nd (0.2 mM) and 3rd groups (0.5 mM) treatments respectively. In the third experiment, semen was divided into three groups. The first group was diluted with Tris only (control group). While the 2nd and 3rd groups were added 0.5 and 1.0 mM α-lipoic acid respectively. The effect of these additions on Holstein bulls semen quality was studied during different periods (48 hours, one, two and three months post cryopreservation) for three experiments. The results revealed that the addition two levels of the $MnCl_2$ (2nd and 3rd, experiment 1), Co- Q10 (2nd and 3rd, experiment 2) and α- lipoic acid (2nd and 3rd experiment 3) led to significant increases freezability as compared with control groups during all the experiment periods. In conclusion, the addition of $MnCl_2$, Co-Q10 and α-lipoic acid led to improved post-cryopreservation semen quality of Holstein bulls. This will in turn enhance fertility rate of artificially-inseminated cows and owners economic income consequently.

Keywords: Anti-oxidant, Manganese chloride, Co-enzyme Q10, α-lipoic acid, Holstein bulls semen and freeze ability.

Introduction

The improvement in quality of semen is an important aspect for maximum utilization of genetically superior sub-fertile sires. The reduction and loss in viability and fertility can be attributed to many changes take place in the sperm cells during cryopreservation (1). Cryopreservation affects the lipid architecture of plasma membrane (2) and metabolism due to high amount of polyunsaturated fatty acids (PUFAs) with significantly less cytoplasmic components containing antioxidants (3). The PUFAs plays an important role in regulating sperm membrane fluidity and spermatogenesis (4). Membrane fluidity plays a great role in regulating ion pump that controls inwards and outwards movement of calcium ion into the spermatozoa. Alteration of the membrane fluidity during freezing-thawing processes will cause accumulation of calcium ion which consequently impaired sperm motility and eventually endanger the sperm survival (5). Bovine sperm themselves have only few amounts of endogenous antioxidants for the protection against reactive oxygen species (ROS) and the main antioxidant source is the seminal plasma (6). Several ROS, produced by both sperm and leukocytes contaminating the seminal fluid, adversely affect sperm motility and impair fertilizing ability. However, low levels of ROS are important for sperm functioning (7). Anti-oxidative mechanisms protect the sperm from the damage caused by free radicals (8). The $MnCl_2$ is an important cofactor of mitochondrial superoxide dismutase (SOD), an antioxidant enzyme

which scavenges oxygen free radicals (9). It inhibits LPO produced by a free radical producing system, as potent antioxidant against oxidative stress at low doses (10). $MnCl_2$ supplementation reduced the leakage of lipids and phospholipid sperm contents under normal and induced oxidative stress conditions (11). Accordingly, Mn^{+2} protects the sperm from the loss of freezing and thawing procedures as it is able to enter the cell more easily and help sperm to maintain or recover appropriate ion balance and thus suffer less from the freezing and thawing procedures (12). Moreover, (13) suggest the role of Mn^{+2} supplementation in improving the quality of bull semen by its scavenging property, through reduction of ROS production during its storage at 4°C or incubation at 37°C for capacitation.

Coenzyme Q10 (Co-Q10), is a fat-soluble molecule which is natural endogenously synthesized antioxidant in all humans and animals. Co-Q10 is an antioxidant, an energy promoting agent, a membrane stabilizer and a regulator of mitochondrial permeability transition pores (14). Moreover, Co-Q10 has enabled to quench the free radicals deteriorative effects and thus preventing lipid and protein peroxidation (15). While, Co-Q10 is capable of reducing α-tocopheroxyl radical to α-tocopherol. This ability of eliminating pro-oxidant radical and regenerating vitamin E will render the hyper functioning of antioxidant in the spermatozoa environment (16). It is noted that Co-Q10 is concentrated in the sperm midpiece region of men (17). Addition of 1µM Co-Q10+5mM α-tocopherol to stallion semen extender improved 48 hrs. post-cryopreservation sperm quality (16).

Alpha-lipoic acid, is a necessary cofactor for mitochondrial α-ketoacid dehydrogenases, and thus serves a critical role in mitochondrial energy metabolism (18). α-lipoic acid has been proved to be a potent antioxidant and capable of scavenging ROS (19). α-lipoic acid has also the potential to regenerate other antioxidants such as vitamins C and E (19). In spite of these promising results, using of α-lipoic acid as semen cryoprotectant is not common confined to specific studies in stallion (20), male buffalo (21) and cattle bulls (22). Thus, it is unable to literature that profoundly evaluates the potential effect on frozen\thawed spermatozoa in Holstein bulls. Limited data have obtained with the addition of $MnCl_2$, Co-Q10 and α-lipoic acid to semen extenders, and their influence on post-cryopreservation semen quality of Holstein bulls. However, the previous studies did not identify the accurate concentrations of these relevant antioxidants, and contradictory results have been reported. Higher concentrations of $MnCl_2$, Co-Q10 and α-lipoic acid may give better post-cryopreservation semen characteristics of Holstein bulls. Therefore, the objective of this study was to investigate effect of adding $MnCl_2$, Co-Q10 and α-lipoic acid to Tris extender on freeze ability of Holstein bulls semen.

Materials and Methods

This study was carried out at the Department of Artificial Insemination at Directorate of Animal Resource, Ministry of Agriculture, Abu-Ghraib, Baghdad for the duration from October\ 2013 to Jun\ 2014 including three experiments. Seven Holstein bulls, 3.5- 4 years old and 500-750 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 were vaccinated with FMD vaccine (Tri-strain Dutch, Intervet, Boxmeer, Holland and Aftovac, Turkey).

Semen was collected from seven bulls using artificial vagina at one ejaculated\ bull\ week. One milliliter of semen was taken from each bull and combining together as pooled semen to remove the individual variations among bulls. Individual motility was evaluated in fresh semen. Pooled semen divided equally to the three different treatments thereafter within each experiment using Tris extender.

Experiment 1: The Effect of adding manganese chloride to Tris extender on freeze ability of Holstein bulls semen. This experiment was undertaken to determine the effect of manganese chloride to Tris extender on freeze ability of Holstein bulls semen post-cryopreservation (PC) (48 hrs., 1st, 2nd and 3rd month). Following collection, semen was divided equally to three groups. The first group was considered as a control group, 1st sub group (A1) was diluted with Tris only. Manganese chloride (Gailad Chemical

Company\ UK) was added to 2nd sub group (A2), 0.7 mM and 3rd sub group (A3), 0.9 mM treatments respectively. Sperm freeze ability was determined according to (23) procedure based on the following equation:

$$\textit{Freeze ability (\%)} = \frac{\textit{percentage of individual motility post thawing}}{\textit{percentage of individual motility fresh}} \times 100$$

Experiment 2: The Effect of adding co-enzyme Q10 to Tris extender post-cryopreservation on freeze ability of Holstein bulls semen. This experiment was conducted to estimate the influence of co-enzyme Q10 (Co-Q10) to Tris extender on freeze ability of Holstein bulls semen PC (48 hrs., 1st, 2nd and 3rd month). Following collection, semen was divided equally to three groups. The first group was considered as a control group, 1st sub group (B1) was diluted with Tris only. Co-enzyme Q10 was added to 2nd sub group (B2), 0.2 mM and 3rd sub group (B3) 0.5 mM treatments respectively. The estimated semen characteristics were as previously mentioned in experiments 1.

Experiment 3: The Effect of adding α-lipoic acid to Tris extender post-cryopreservation on freeze ability of Holstein bulls. This experiment was undertaken to investigate the influence of α-lipoic to Tris extender on freeze ability of Holstein bulls semen PC (48 hrs., 1st, 2nd and 3rd month). Following collection, semen was divided equally to three groups. The first group was considered as a control group, 1st sub group (C1) was diluted with Tris only. α-lipoic acid was added to 2nd sub group (C2) 0.5 mM and 3rd sub group (C3) 1.0 mM treatments respectively. Semen evaluation was done on each treatment after 48 hours, 1st, 2nd and 3rd month PC. The estimated semen characteristics were as previously mentioned in experiments 1.

The statistical computations were performed using SAS program (24) based on completely randomized design to study the effect of different factors on the studied characteristics. Means with significant differences were compared using Duncan multiple range test (25). The two statistical models were as follows:

The 1st statistical model for comparison among groups within each preservation time. $Y_{ij} = \mu + T_i + e_{ij,}$ The 2nd statistical models for comparison among periods within each treatment, $Y_{ij} = \mu + Pi + eij$.

Results and Discussion

Experiment 1: Statistical analysis showed highly ($P \leq 0.0001$) significant effect of two manganese chloride doses (A2 and A3) on FA% among different preservation periods as compared with A1 group (Table, 1).

Forty eight hours PC recorded higher ($P \leq 0.0001$) FA% than 1st, 2nd and 3rd month PC within A1 and A2 groups (Table, 1). Meanwhile, no differences were noticed among 1st, 2nd and 3rd month PC in FA% within A1 (49.50±1.76 - 52.39±1.48 %) and A3 (66.05±2.1 - 70.38±0.82 %) groups (Table, 1). Forty eight hours (76.74±1.11%) and 1st month (69.32±1.24%) PC observed greater ($P \leq 0.0001$) difference in FA% as compared with both 2nd and 3rd (62.93±1.16%) (Table,1).

Individual motility result was in line with (12) who found that the addition of 0.1 mM $MnCl_2$ to semen extender improved PC individual motility (53%) in comparison with control group (28%). The current results also agreed with (26) who found that the addition of $MnCl_2$ (150 µM) to egg-yolk-citrate extender increased PS individual motility (58.3%) as compared with the control group (45%). However, the current $MnCl_2$ concentrations are higher than those pointed out in the previous studies. Many preliminary trials were conducted to select these concentrations based on their significant effects. It is worthy to mention that the concentrations used by the previous studies had negative effects on PC semen characteristics when used in the preliminary trials. Therefore, it is the first study that describes the effect of high $MnCl_2$ concentrations (0.7 and 0.9 mM) as added to Tris extender on post-cryopreservation semen quality in Holstein bulls.

Manganese is an important cofactor of mitochondrial superoxide dismutase antioxidant enzyme which scavenges oxygen free radicals (26 and 27). Mn^{+2} may stimulate the enzymes of glutathione cycle and affect the total thiols, glutathione reduced and glutathione oxidized contents in bull spermatozoa (11). It acts as a potent

antioxidant in protection against OS (28). Currently, Mn^{+2} enhanced freeze ability might return to stimulate the activity of sperm adenylate cyclase enzyme to maximum extent (29). Manganese is a potent stimulator of adenylate cyclase activity in the sperm cells, and cyclic adenosine monophosphate (cAMP) concentrations are correlated with the cell motility (12). Other researchers (30) have described that energy transduction within sperm tail, made possible by the molecular diffusion of mitochondrial adenosine triphosphate (ATP) along the flagellum for rhythmic flagellar movements. This energy, according to these workers is generated along the flagellum by a mechanic-chemical process coupled to enzymatic dephosphorylation of ATP. However, this ATP dephosphorylation is not entirely irreversible. Some of the dephosphorylated ATP can be resynthesized as a result of axonemal adenylate kinase activity. Manganese has also proved to be the best antioxidant in reducing the ferrous ascorbate-induced LPO in bull spermatozoa (31). Mn^{+2} protects membrane from peroxidative damage produced by the superoxide radicals (O_2^{-}). In conclusion, addition of $MnCl^2$ (0.7 and 0.9 mM) to Tris extender inhibits LPO, and thus improving the sperm membrane integrity and the remaining semen characteristic at PC periods. This will certainly lead to enhance fertility of artificially- inseminated cows.

Experiment 2: Greater ($P\leq0.0001$- $P\leq0.001$) effect of B2 and B3 group was noticed on the FA% among different preservation periods as compared with a B1 group (Table, 2). Forty eight hours PC recorded higher ($P\leq0.0001$- $P\leq0.001$) FA% than 1st, 2nd and 3rd month PC within B1, B2 and B3 groups. Meanwhile, no significant differences were noticed among 1st, 2nd and 3rd month PC in FA% within B1 (49.50±1.76 - 52.39±1.48%) and B2 (60.2 ± 2.29-64.79 ± 2.15%) groups (Table, 2). First month (69.22 ± 2.02%) PC observed greater difference ($P\leq0.0001$) in FA% as compared with both 2nd and 3rd (62.42 ± 2.14%) (Table, 2).

The addition of two CoQ10 levels (B2 and B3) enhanced sperm motility and FA. This may due to the essential role of CoQ10 in ATP production. ATP synthesis and energy generation is related to the availability and existence of Co-Q10 in the sperm cell (32). The possible explanation is the involvement of the Co-Q10 in the regulation of energy production as it is concentrated within the mitochondrial midpiece (17). Furthermore, Co-Q10 is an integral redox and proton translocating component of the mitochondrial respiratory chain (32). Since soluble adenylyl cyclase (sAC) which is vital for sperm motility activation is also confined to the midpiece region, it is possible for this highly lipophilic antioxidant to diffuse and directly protect the sAC. Subsequently, it will influence the pattern of sperm progressive motility (22). Moreover, Co-Q10 is also capable of reducing alpha-tocopheroxyl radical to alpha-tocopherol. This ability of eliminating pro-oxidant radical and regenerating vitamin E will render the hyper functioning of antioxidant in the spermatozoa environment (15). Co-Q10 exhibits both antioxidative and membrane stabilizing property and helps to prevent sperm cells from damage caused by ROS and other free radicals, preventing LPO in sperm plasma membrane (33 and 34) and maintained sperm plasma membrane integrity (34). As Co-Q10 is highly lipophilic, so that it can diffuse the phospholipid bilayer of cellular membrane and this protect the sperm plasma membrane.

In conclusion the adding of 0.5 mM Co-Q10 to Tris extender inhibits LPO, thus increasing the membrane integrity and all characteristic of semen at cooling and PC. Higher fertility and pregnancy rates were expected of artificially-inseminated cows resulting from improved PC semen traits. The Co-Q10 is one of the substantial future antioxidant that may use in artificial insemination centers in Iraq and worldwide due to its beneficial effects. Commercial artificial insemination will inevitably use this treatment to preserve and transport semen over a wider area around the world. However, other studies are needed using higher concentrations of Co-Q10 and its effects on PC semen traits of bulls and other livestock species.

Experiment 3: Higher significant ($P\leq0.0001$) effect of two α- lipoic acid levels (C2 and C3) were noticed on FA% among different preservation periods as compared with C1 group (Table, 3). Within each group, 48 hrs. PC recorded higher ($P\leq0.0002$-

P≤0.01) FA% than 1st, 2nd and 3rd month PC (Table, 3). Meanwhile, no significant differences were noticed in FA% among 1st, 2nd and 3rd month PC within C1 (49.30±1.22 - 51.92±2.23 %), C2 (64.05±1.53 - 68.46±1.89 %) and C3 (61.45±2.64 - 63.74±3.35 %) groups (Table, 3).

Table, 1: The Effect of adding manganese chloride to Tris extender on the freeze ability percentage (FA%) at different post-cryopreservation (PC) periods in Holstein bulls semen (M ±S.E).

Period Treatment	48 Hours PC	First month PC	Second month PC	Third month PC	Level of significance
A1	63.37 ± 0.97 B a	52.39 ± 1.48 B b	50.75 ± 1.73 B b	49.50 ± 1.76 B b	P≤0.0001
A2	76.74 ± 1.11 A a	69.32 ± 1.24 A b	62.93 ± 1.16 A c	62.93 ± 1.16 A c	P≤0.0001
A3	76.69 ± 1.38 A a	70.38 ± 0.82 A b	66.04 ± 2.1 A b	66.05 ± 2.1 A b	P≤0.0001
Level of significance	P≤0.0001	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.
A1: Tris extender (Control group). A2: Tris extender + 0.7 mM manganese chloride. A3: Tris extender + 0.9 mM manganese chloride.

Table2. The Effect of adding Co-Q10 to Tris extender on the freeze ability percentage (FA%) different post-cryopreservation (PC) periods in Holstein bulls semen (M ±S.E).

Period Treatment	48 Hours PC	First month PC	Second month PC	Third month PC	Level of significance
B1	63.37 ± 0.97 B a	52.39 ± 1.48 B b	50.75 ± 1.73 B b	49.50 ± 1.76 B b	P≤0.0001
B2	72.78 ± 2.18 A a	64.79 ± 2.15 A b	61.39 ± 2.43 A b	60.2 ± 2.29 A b	P≤0.002
B3	77.22 ± 1.99 A a	69.22 ± 2.02 A b	62.42 ± 2.14 A c	62.42 ± 2.14 A c	P≤0.0001
Level of significance	P≤0.0001	P≤0.0001	P≤0.001	P≤0.0007	

Means with different capital superscripts within each column indicate comparison among treatments and small superscripts within each row indicate comparison among periods within each treatment.
B1: Tris extender (Control group). B2: Tris extender + 0.2 mM Co-Q10. B3: Tris extender +0.5 mM Co-Q10.

Table, 3: The Effect of adding α- lipoic acid to Tris extender on the freeze ability percentage (FA %) at different post-cryopreservation (PC) periods in Holstein bulls semen (M ±S.E).

Period Treatment	48 Hours PC	First month PC	Second month PC	Third month PC	Level of significance
C1	62.24±1.87 B a	51.92±2.23 B b	50.73±1.96 B b	49.30±1.22 B b	P≤0.0002
C2	74.99±1.29 A a	68.46±1.89 A b	66.17±1.84 A b	64.05±1.53 A b	P≤0.0006
C3	73.35±2.3 A a	63.74±3.35 A b	61.45±2.64 A b	61.45±2.64 A b	P≤0.01
Level of significance	P≤0.0002	P≤0.0008	P≤0.0003	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.

Addition of 0.5 mM and 1 mM α-lipoic acid had an overwhelming effect (P≤0.01) in improving IM and FA, as compared with the control group during all the experimental periods. Some of the current results were in line with (20) who pointed out that the sperm motility rate and viability were improved following incubation with α-lipoic acid at a concentration of 0.025 mmol\ ml. This concentration was also capable of reducing DNA damage. The current results were also agreed with (21) who observed that addition of 0.5 and 1mM α-lipoic acid to Tris extender improved PC IM of buffalo semen. The current α-lipoic acid concentrations are higher than those observed by (20) in Malaysia. Preliminary trials were conducted to select these concentrations based on their effective action. It is noteworthy that the concentration used by (20) had non-significant effects on PC semen characteristics when used in the preliminary trials. Therefore, it is the first

study that describes the effect of high α-lipoic acid concentrations (0.5 and 1 mM) as added to Tris extender on PC semen quality in Holstein bulls. Furthermore, the C2 group (0.5 mM) exhibit greater PC semen improvements that C3 group (1 mM) that achieved limited enhancements.

The Addition of α-lipoic acid (0.5 mM) to Tris extender improved most of the current PC semen characteristics. This may be due to a potent biological antioxidant and a detoxification role of α-lipoic acid (18). The α-lipoic acid has assisted in the metabolism of oxidative decarboxylation by acting as a co-enzyme (35). 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 (36). 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 (37). 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 (38). The oxidized and reduced α-lipoic acid forms create a potent redox couple that has a standard reduction potential. Reduced α-lipoic acid is one of the most potent naturally occurring antioxidants. Both oxidized and reduced α-lipoic acid may scavenge hydroxyl radicals and hypochlorous acid as well as terminates singlet oxygen (35). Neither species is active against hydrogen peroxide (35). Furthermore, reduced α-lipoic acid appears to regenerate other endogenous antioxidants (e.g. vitamins C and E) (39) and has the salubrious property of neutralizing free radicals without itself becoming one in the process.

In conclusion, addition of α-lipoic acid (0.5 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. Simultaneously, other studies are warranted to confirm these results either in bulls of other livestock species.

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تأثير إضافة كلوريد المنغنيز والمرافقات الإنزيمية (Q10 و α- lipoic acid) في الصفات النوعية للسائل المنوي لثيران الهولشتاين بعد الحفظ بالتجميد

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الخلاصة

أجريت هذه الدراسة بهدف بيان تأثير اضافة كلوريد المنغنيز والمرافق الانزيمي Q10 وحامض الالفا ليبويك إلى مخفف ترس في قابلية تجميد السائل المنوي لثيران الهولشتاين بعد الحفظ التجميد لمدد مختلفة. أجريت هذه الدراسة في قسم التلقيح الاصطناعي التابع لدائرة الثروة الحيوانية- وزارة الزراعة في أبي غريب/بغداد للمدة من شهر تشرين الأول/2013 وحتى شهر حزيران/2014 وبواقع ثلاث تجارب. استعمل في هذه الدراسة سبعة ثيران هولشتاين بأعمار تتراوح بين 3.5–4 سنوات. جمع السائل المنوي بوساطة المهبل الاصطناعي بواقع قذفة واحدة/ ثور/ أسبوع. أجريت الفحوص اللازمة لتقييم السائل المنوي الطازج والذي جُمع لاحقاً للثيران جميعها وقُسم بالتساوي على المعاملات المختلفة في التجربة الواحدة باستعمال مخفف ترس. تم في التجربة الأولى تقسيم السائل المجموع إلى ثلاث مجاميع بالتساوي، اذ عدت المجموعة الأولى مجموعة سيطرة بإضافة مخفف ترس فقط، في حين أضيف كلوريد المنغنيز إلى المعاملة الثانية (0.7 مليمول) في حين في المجموعة الثالثة (0.9 مليمول) أضيف من كلوريد المنغنيز. قسم السائل المنوي في التجربة الثانية إلى ثلاثة مجاميع متساوية، إذ عدت المجموعة الأولى مجموعة سيطرة بإضافة مخفف ترس فقط، في الوقت الذي أضيف فيه المرافق الانزيمي Q10 إلى كل من المجموعة الثانية (0.2 مليمول) والمجموعة الثالثة (0.5 مليمول) على التوالي. قسم السائل المنوي في التجربة الثالثة إلى ثلاثة مجاميع متساوية، اذ كانت المجموعة الاولى بمثابة مجموعة سيطرة بإضافة مخفف ترس فقط، في حين اضيف 0.5 و 1.0 مليمول من الالفا ليبويك إلى مخفف ترس في المجموعتين الثانية والثالثة على التوالي. دُرس تاثير هذه الإضافات في صفات السائل المنوي في مدد حفظ زمنية مختلفة (بعد 48 ساعة وشهر وشهرين وثلاثة اشهر) للتجارب الثلاثة. أوضحت النتائج أن إضافة مستويين من كلوريد المنغنيز في التجربة الأولى (المجموعة الثانية والثالثة) والمرافق الانزيمي Q10 (المجموعة الثانية والثالثة من التجربة الثانية) والالفا ليبويك (المجموعة الثانية والثالثة من التجربة الثالثة) أدت إلى زيادة معنوية في النسبة المئوية لقابلية النطف على التجميد مقارنة بمجموعة السيطرة خلال مدد حفظ التجميد جميعها. يمكن الإستنتاج بأن إضافة مركبات كلوريد المنغنيز والمرافق الإنزيمي Q10 والألفا ليبويك إلى مخفف ترس أدى إلى تحسين نوعية السائل المنوي بعد الحفظ بالتجميد لثيران الهولشتاين، وهذا قد يساهم في زيادة نسبة الخصوبة لدى الأبقار ومن ثم زيادة العائد الاقتصادي للمربي.

الكلمات المفتاحية: مضادات الاكسدة، كلوريد المنغنيز، الفا ليبويك اسيد، المرافق الانزيمي Q10، السائل المنوي للثيران الهولشتاين وقابلية التجميد.