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EFFECT OF ADDING CO-ENZYME Q10 TO TRIS DILUENT ON SEMEN QUALITY OF BULLS AT DIFFERENT PRESERVATION PERIODS

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ABSTRACT

This study evaluated how adding coenzyme Q10 to the Tris diluent affects semen quality in Holstein bulls after cooling and cryopreservation at 2, 30, 60, and 90 days. Each week, semen was collected from each bull using an artificial vagina and then pooled. The pooled semen was split into three groups: the control group (Q1) received only Tris, while the other two groups had coenzyme Q10 added to the Tris diluent at 0.2 mM (Q2) and 0.5 mM (Q3). The results showed that adding 0.5 mM Co-Q10 significantly increased the total number of progressive motile spermatozoa (×106), the percentage of sperm with normal morphology, plasma membrane integrity, overall sperm function, and acrosome integrity compared with the control groups throughout all experimental periods. In conclusion, the addition of 0.5 mM Co-Q10 improved the post-cryopreservation semen quality of Holstein bulls. This enhancement is expected to increase the fertility rate of artificially inseminated cows and, in turn, support economic growth. Enhanced semen quality will contribute to higher animal productivity, helping to meet growing consumption demands, address the rising population, and adapt to the climate changes currently affecting the planet.

Key words: **antioxidant; cryopreservation; semen extender; sustainable economic growth.**

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INTRODUCTION

The world is witnessing population growth and climate change. This requires utilizing all agricultural outputs, directing them to increase agricultural productivity and address population growth to ensure decent work, sustainable economic growth, and sustainable production and consumption (UNDESA, 2021; Hemathilake and Gunathilake, 2022; Alwaeli et al., 2024). Bovine semen cryopreservation is essential for commercial artificial insemination. Although this process is widely practiced and has advanced more than the freezing of sperm from other species, challenges remain (Upadhyay et al., 2021; Sharafi et al., 2022). The cryopreservation and disruption of sperm plasma membrane configuration due to changes in metabolic pathways, enzymes, and antioxidant activity add to lower efficiency with loss of sperm longevity and fertilizing ability (Upadhyay et al., 2021; Sharafi et al., 2022; Ibrahim, 2024; Castro et al., 2025). Cryopreserved bull semen has a shorter lifespan and lower fertility than fresh semen (Longobardi et al., 2020). These differences are partly due to variations between fresh and frozen sperm in the generation rates of O2−• and H2O2, and in the intracellular concentration of free Ca^{+2} (Gualtieri et al., 2021). Antioxidants effectively neutralize reactive oxygen species (ROS). Supplementing bull semen diluent with antioxidants significantly increased sperm motility, viability, normal morphology, plasma

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membrane integrity, and ATP activity (Ahmed et al., 2022). Oxidation of thiols in sperm proteins correlates with reduced sperm motility and fertilizing capacity (Vigolo et al., 2022; Nakao et al., 2025; Onochie et al., 2025). Ultrastructural analyses have demonstrated that cryopreservation adversely affects multiple sperm organelles, including mitochondria and plasma membranes, and increases sperm mortality (Sun et al., 2020; Gonzalez et al., 2022). Swelling of the sperm acrosomal region has been observed following cold shock, indicating loss of integrity (Nixon et al., 2021; Esin and Kaya, 2025). Additionally, freezing alters spermatozoa motility due to irreversible changes in the mid-piece and tail coiling (Sun et al., 2020; Dementieva et al., 2024). Iraq suffers from climate changes represented by global warming, high temperatures, lack of rain, and the availability of fodder even in moderate seasons (Alwaeli and Eidan, 2024). The bull's semen quality for artificial insemination has decreased, which leads to a decrease in the production of semen straw for artificial insemination, as well as affecting the sustainability of production and consumption for citizens. In order to confront this deterioration of semen quality, many studies have been conducted to improvement semen, including the detection of genetic mutations of genes (Sultan and Eidan, 2020; Precone et al., 2021; Liang et al., 2024), metabolic changes (Musa and Abdulkareem, 2023), sperm filtration (Alwaeli and Eidan, 2023; Fleming et al., 2025) and antioxidants (Eidan et al., 2020; Shepherd et al., 2024; Younus et al., 2024; Loetjettanarom et al., 2025). Co-enzyme Q10 (Co-Q10) is a fat-soluble, vitamin-like molecule naturally produced that act as a non-enzymatic antioxidant linked to low-density lipoproteins (Silva et al., 2022; Abhinaya et al., 2025). Co-Q10 widely distributed throughout the cell body (Suárez-Rivero et al., 2021; Mantle et al., 2024). Coenzyme Q10 (Co-Q10) functions as an energy-promoting agent, membrane stabilizer, and regulator of mitochondrial permeability transition pores (Suárez-Rivero et al., 2021). Recent studies have demonstrated that Co-Q10 is concentrated in the midpiece region of spermatozoa (Lançoni et al., 2021). Therefore, Co-Q10 deficiency may contribute to reduced sperm motility in some men. A strong correlation between sperm count, motility, and Co-Q10 content in human seminal fluid has been reported (Alleva et al., 1997; Akhigbe et al., 2025). CoQ10 can be used for treating men asthenozoospermic infertility with the dosage and duration depending upon the severity of the disorder and the patient's response to the treatment (Vishvkarma et al., 2020). Coenzyme Q10 (Co-Q10) concentration in seminal fluid correlates directly with semen parameters (Lewin and Lavon, 1997; Mancini et al., 2005; Bakri et al., 2025). Co-Q10 enhances sperm motility and viability by protecting against reactive oxygen species (ROS) damage and preventing lipid peroxidation in the sperm plasma membrane (Salvio et al., 2021). Eidan et al. (2017) found an addition of Co-Q10 led to improve semen freezability of Holstein bulls. This study investigated the effect of adding Co-Q10 to semen diluent on Holstein bulls' semen quality after cooling and cryopreservation.

MATERIALS AND METHODS

The study was undertaken at the Department of Artificial Insemination, Directorate of Animal Resources, Ministry of Agriculture. Eight Holstein bulls, weighing between 500 and 750 kg and aged 3.5 to 4 years, were trained for semen collection using an artificial vagina. All experimental bulls were healthy and disease-free, and were continuously monitored by a veterinarian. Bulls received a daily concentrate ration of 4 kg per head (3323 kcal/kg and 18% crude protein) along with 50 to 60 kg of green fodder per bull. This study aimed to evaluate the effect of co-enzyme Q10 (Co-Q10) added to Tris diluent after cooling (PCO; 5 degrees Celsius for 5 hours) and cryopreservation (PCY) on semen characteristics. Semen was collected weekly from seven bulls (one ejaculate per bull per week) and pooled over an 8-week period. Semen samples were divided equally into three treatment groups. The first group served as the control treatment (Q1) and was diluted solely with Tris. The 0.2 and 0.5 mM Co-enzyme Q10 was added to Tris diluent in Q2 () and Q3 treatments respectively. Semen was packed in

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straws (20 million sperm/ straw) and preservation in liquid nitrogen (-196º C). Semen evaluation was conducted for each treatment, involving 20 straws per trait per week for PCO and PCY at 2, 30, 60, and 90 days. The total number of progressively motile sperm, plasma membrane integrity, normal morphology, functional sperm fraction, and acrosomal integrity of bulls' spermatozoa were assessed according to the methods described by Srivastava and Pande (2017). Statistical analyses were conducted using SAS within a completely randomized design (CRD) to examine the effects of various factors on the measured characteristics. Means exhibiting significant differences were compared using the Duncan multiple range test.

RESULTS AND DISCUSSION

The total number of progressive motile spermatozoa (TPMS×106) per straw was significantly higher ($p \leq 0.01$) in the Q2 (7.57-10.85) and Q3 (7.85-13.94) treatments compared to the Q1 treatment (5.42-9.16) across various preservation periods (Table 1). Q2 and Q3 significantly affected total plasma membrane integrity of bull spermatozoa (TPMIBS; ×106) under various preservation conditions compared to Q1 ($p \leq 0.01$, Table 2). Furthermore, in TPMIBS, the differences between Q1 and Q2 treatments at cooling lacked significance (Table 2). There were no significant differences between Q3 and Q2 treatments at different PCY (Table 2). The Q3 exhibited a clear difference ($p \leq 0.01$) in the total normal morphology of bull spermatozoa (TNMBS; ×106) in each straw as compared to treatment at different preservation periods (Table 3). Significant differences ($P \leq 0.01$) in TNMBS were observed between Q2 and Q1 treatments at the first month post-cooling (PC). No significant differences were detected between Q2 and Q3 across preservation periods, except during cooling, which exhibited substantial differences ($P \leq 0.01$) (Table 3). The Q3 and Q2 treatments exhibited higher ($p \leq 0.01$) total function sperm fraction (TFSF; ×106) numbers/ straw than the Q1 treatment at all preservation periods (Table 4). On the other hand, Q3 treatment revealed higher ($p \leq 0.01$) TFSF numbers than Q2 treatment post-cooling and 2 days post cryopreservation periods (Table 4). Non-significant differences were noticed for the total acrosome integrity of bull spermatozoa (TAIBS; ×106) between Q1 and Q2 treatments at different preservation periods (Table 5). The Q3 exhibited the highest ($p \leq 0.01$) TAIS value than both Q1 and Q2 treatments at overall preservation periods (Table 5). The addition of Co-Q10 at 0.5 mM (Q3) significantly improved TPMS, TPMIBS, TNMBS, TFSF, and TAIBS compared to control treatments across various preservation periods ($p < 0.01$). Additionally, supplementing the Tris diluent with 0.2 mM Co-Q10 (Q2) enhanced TMS, TPMIS, and TFSF relative to controls ($P \leq 0.05$). These findings align with Ibrahim et al. (2011), who reported a significant increase in bull sperm curvilinear velocity 4 minutes after separation when incubated with 3 µM CoQ10 in Bioxcell® diluent. Additionally, 6 µM CoQ10 significantly improved sperm progressive motility compared to the control. The current results align with Yousefian et al. (2014), who found that supplementing stallion semen in skim milk-based diluent with 1mM Co-Q10 significantly increased total motility (62.44±3.82 vs. 54.44±3.63%, $P < 0.05$) and plasma membrane integrity (65.16±3.63 vs. 38.87±3.75%) compared to the negative control after 48 hours of cooling storage. Our results agreed with the results of Akhigbe et al. (2025), who mentioned increases in total and progressive sperm motility and normal morphology in male supplementation CoQ10. Adding two CoQ10 concentrations (Q2 and Q3) enhanced sperm motility, likely because CoQ10 supports ATP production. Co-Q10 is effective in preserving mitochondria functionality and the cytoskeleton of sperm cells submitted to the cryopreservation process (Lançoni et al., 2021; Farshad and Wehrend, 2025). ATP synthesis and energy production in sperm cells depend on the availability of Co-Q10 (Lewin and Lavon, 1997; Ahmed et al., 2023,). Energy production depends on the normal operation of the electron transport chain in mitochondria. The CoQ10 plays a central role in the electron transport chain. The electron transport chain is composed of four protein complexes in addition to ATP synthase to produce ATP (Nolfi-Donegan et al., 2020;

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Nie et al., 2023). Co-Q10 is likely involved in energy production due to its concentration in the mitochondrial midpiece (Alahmar and Sengupta, 2021; Sumbalová et al., 2025; Zamil et al., 2025). It is also a key redox and proton-translocating component of the mitochondrial respiratory chain (Lewin and Lavon, 1997; Pallotti et al., 2021). Cyclic AMP is one of the most important intracellular factors in the sperm signaling pathway. Adenylyl cyclase is the key enzyme that synthesizes cyclic AMP at the onset of the signaling pathway in all cellular functions (Shiba and Inaba, 2023; Li et al., 2024). Soluble adenylyl cyclase is vital for sperm motility activation and is confined to the midpiece region. This highly lipophilic antioxidant can diffuse and directly protect soluble adenylyl cyclase (Chang et al., 2021), this protection, in turn, will influence the pattern of sperm progressive motility (Ibrahim et al., 2011). The addition of Co-Q10 improved TPMS, TPMIBS, TFSF (Q2 and Q3), TNMS (Q3), and TAIS (Q3). This may reflect Co-Q10's antioxidant ability to quench free radicals, thus preventing lipid and protein peroxidation (Rodick et al., 2018; De Barcelos and Haas, 2019; Silva et al., 2022; Abi Nahed et al., 2025; Manful et al., 2025).

Table 1. Effect of Coenzyme Q10 addition to Tris diluent on total progressive motile spermatozoa (× 10^6) in bulls across various preservation periods

Treatment Period	Q1	Q2	Q3	significance
Cooling (5˚ C; 5 hrs.)	9.16 ^{B} ± 0.65	10.85 ^{A} ± 0.34	13.94 ^{A} ± 0.53	P≤0.01
Post-cryopreservation (day):				
2	6.85 ^{B} ± 0.34	9.14 ^{A} ± 0.34	9.71 ^{A} ± 0.42	P≤0.01
30	5.71 ^{B} ± 0.42	8.14 ^{A} ± 0.34	8.71 ^{A} ± 0.42	P≤0.01
60	5.57 ^{B} ± 0.48	7.71 ^{A} ± 0.35	7.85 ^{A} ± 0.40	P≤0.01
90	5.42 ^{B} ± 0.48	7.57 ^{A} ± 0.36	7.85 ^{A} ± 0.40	P≤0.01

Capital superscripts within each row indicate comparisons among treatments
Q1=Semen+ Tris diluent (Control treatment); Q2=Q1 + 0.2 mM Co-Q10; Q3= Q1 +0.5 mM Co-Q10

Table 2. Effect of Co-Q10 supplementation in Tris diluent on total plasma membrane integrity of bull spermatozoa (×10^6) across various preservation periods

Treatment Period	Q1	Q2	Q3	significance
Cooling (5˚ C)	10.71 ^{B} ± 0.52	12.33 ^{B} ± 0.40	13.94 ^{A} ± 0.53	P≤0.01
Post-cryopreservation (day):				
2	10.87 ^{B} ± 0.50	12.46 ^{A} ± 0.57	13.58 ^{A} ± 0.51	P≤0.01
30	10.25 ^{B} ± 0.60	12.73 ^{A} ± 0.48	13.45 ^{A} ± 0.44	P≤0.01
60	9.92 ^{B} ± 0.58	12.17 ^{A} ±0.59	13.22 ^{A} ±0.50	P≤0.01
90	9.8 ^{B} ± 0.53	12.11 ^{A} ±0.48	13.05 ^{A} ±0.52	P≤0.01

Capital superscripts within each row indicate comparisons among treatments. Q1= Semen + Tris diluent (Control treatment); Q2= Q1+ 0.2 mM Co-Q10; Q3=Q1+ 0.5 mM Co-Q10

Table 3. Effect of Co-Q10 supplementation in Tris extender on total normal morphology of bull spermatozoa (×10^6) across various preservation periods

Treatment Period	Q1	Q2	Q3	significance
Cooling (5˚ C; 5 hrs.)	15.55 ^{B} ± 0.11	15.91 ^{B} ± 0.20	16.42 ^{A} ± 0.13	P≤0.01
Post-cryopreservation (day):				
2	15.55 ^{B} ± 0.16	16.01 ^{AB} ± 0.13	16.44 ^{A} ± 0.19	P≤0.01
30	15.51 ^{B} ±0.20	15.99 ^{A} ± 0.13	16.23 ^{A} ± 0.10	P≤0.01
60	15.68 ^{B} ± 0.08	16.09 ^{AB} ± 0.23	16.32 ^{A} ± 0.11	P≤0.01
90	15.41 ^{B} ±0.10	15.87 ^{AB} ± 0.21	16.29 ^{A} ± 0.14	P≤0.01

Capital superscripts within each row indicate comparisons among treatments
Q1= Semen + Tris diluent (Control treatment); Q2= Q1+ 0.2 mM Co-Q10; Q3=Q1+ 0.5 mM Co-Q10

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Table 4. Effect of Coenzyme Q10 addition to Tris diluent on the total functional sperm fraction (×10^6) in bulls across various preservation periods

Treatment Period	Q1	Q2	Q3	Significance
Cooling (5° C; 5 hrs.)	3.79 ^{C} ±0.27	5.31 ^{B} ± 0.22	6.39 ^{A} ± 0.37	P≤0.01
Post-cryopreservation (day):				
2	2.88 ^{C} ±0.18	4.56 ^{B} ± 0.28	5.59 ^{A} ± 0.37	P≤0.01
30	2.25 ^{B} ±0.18	4.16 ^{A} ± 0.27	4.76 ^{A} ± 0.33	P≤0.01
60	2.15 ^{B} ±0.23	3.78 ^{A} ± 0.28	4.26 ^{A} ± 0.32	P≤0.01
90	2.07 ^{B} ±0.25	4.53 ^{A} ± 0.77	4.19 ^{A} ± 0.32	P≤0.01

Capital superscripts within each row indicate comparisons among treatments
Q1= Semen + Tris diluent (Control treatment); Q2= Q1+ 0.2 mM Co-Q10; Q3=Q1+ 0.5 mM Co-Q10

Table 5. Effect of Coenzyme Q10 supplementation in Tris diluent on total acrosomal integrity of bull spermatozoa (×10^6) across various preservation periods

Treatment Period	Q1	Q2	Q3	Significance
Cooling (5° C; 5 hrs.)	13.63^{B} ± 0.24	13.54^{B} ± 0.22	15.08^{A}± 0.33	P≤0.01
Post-cryopreservation (day):				
2	13.05^{B} ±0.22	13.44^{B}± 0.44	14.79^{A} ± 0.25	P≤0.01
30	12.49^{B} ±0.17	12.53^{B} ± 0.41	14.39^{A} ± 0.33	P≤0.01
60	12.48^{B} ±0.21	12.46^{B} ± 0.43	13.86^{A} ± 0.29	P≤0.01
90	12.24^{B} ±0.20	12.12^{B}± 0.36	13.78^{A}± 0.31	P≤0.01

Capital superscripts within each row indicate comparisons among treatments
Q1: Tris diluent (Control treatment); Q2: Tris diluent + 0.2 mM Co-Q10; Q3: Tris diluent +0.5 mM Co-Q10

Co-Q10 can reduce alpha-tocopherol radical to alpha-tocopherol (Lass et al., 1999. Sifuentes-Franco et al., 2022). This ability to neutralize pro-oxidant radicals and regenerate vitamin E may enhance antioxidant activity in the spermatozoa environment (Tippairote et al., 2022; Wang et al., 2025). Coenzyme Q10 is especially present in high concentrations in the mitochondria of sperm, where it is involved in cell respiration and plays an integral role in energy production (Lançoni et al., 2021; Fadhil and Althanoon, 2025). This positive effect promotes its use as a motility stimulant and antioxidant molecule. Interestingly, CoQ10 inhibits the formation of superoxide, protecting against oxidative stress-induced sperm dysfunction (Kowalczyk, 2022; Alahmar et al., 2023; Chen et al., 2026). Co-Q10 has anti-oxidative and membrane-stabilizing properties that protect sperm cells by neutralizing reactive oxygen species (ROS) and other free radicals. It prevents lipid peroxidation in the sperm plasma membrane, enhancing cell-freezability (Eidan et al., 2017; Alahmar and Sengupta, 2021) and preserving membrane integrity. Co-Q10 is highly lipophilic, so it can diffuse the phospholipid bilayer of the cellular membrane, protecting the sperm plasma membrane (Littarru and Tiano, 2007; Appiah et al., 2020). The improvement of total plasma membrane integrity in current results in Q2 and Q3 treatment may be due to the role of Co-Q10 in combatting ferroptosis. Extra mitochondrial CoQ has been implicated in combatting ferroptosis, a form of nonapoptotic cell death caused by iron-dependent phospholipid peroxidation (Dixon et al., 2012; Guerra and Pagliarini, 2023). Oxidative stress from environmental and metabolic stimuli can catalyze the formation of toxic phospholipid hydroperoxides from polyunsaturated fatty acids, resulting in a loss of membrane integrity, oxidative damage to macromolecules, and cell death (Jiang et al., 2021; Guerra and Pagliarini, 2023). Accordingly, improved SPMI, IM, AI, TMS, TPMIS and TAIS were noticed in Q2 and Q3 treatments as compared with Q1 treatment.

CONCLUSION

It can be concluded that adding Co-Q10 (0.5 mM) to the Tris diluent inhibits lipid peroxidation. This inhibition increases membrane integrity and improves semen traits throughout various preservation periods. This leads to the high fertility expected of

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artificially inseminated cows, which is reflected in increased animal production to meet the population increase and climate changes that the Earth is witnessing. So, due to its beneficial effects, Co-Q10 is a promising future antioxidant that may be used in artificial insemination centers. Commercial artificial insemination will likely use it to help preserve and transport semen over greater distances. However, more studies are needed with higher Co-Q10 concentrations to see its effects on bulls' semen after freezing.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this study.

DECLARATION OF FUND

The authors declare that no funding was received for this study.

AUTHOR/S DECLARATION

We confirm that all figures and tables presented in this manuscript are original. Any figures or images not produced by the authors have been included with the necessary permissions for republication, and documentation of these permissions has been provided with the manuscript.

- The author(s) have signed the Ethical Approval Statement.

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تأثير أضافة المرافق الانزيمي Q10 الى مخفف الترس في نوعية السائل المنوي للثيران خلال أوقات حفظ مختلفة

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المستخلص

قيّمت هذه الدراسة تأثير إضافة المرافق الانزيمي Q10 إلى مخفف Tris في صفات السائل المنوي بعد الحفظ بالتبريد والتجميد لثيران الهولشتاين عند اوقات مختلفة (2، 30،60،90 يوم). تم جمع السائل المنوي بواسطة المهبل الاصطناعي (قذفة/الثور/الأسبوع) وتم تجميعه. قُسِمَ السائل المنوي بالتساوي إلى ثلاث معاملات، تم تخفيف المجموعة الأولى باستخدام مخفف Tris فقط (معاملة السيطرة، Q1)، في حين تم إضافة المرافق الانزيمي Q10 إلى مخفف Tris في المعاملتين الثانية (0.2 مليمول، Q2) والثالثة (0.5 مليمول، Q3). أظهرت النتائج أن إضافة 0.5 مليمول من Co–Q10 أدت إلى زيادة العدد الكلي ($10^6\times$) للحركة التقدمية للنطف والنطف الطبيعية والسليمة الغشاء البلازمي، والعدد الكلي لوظائف النطف الحيوية والسليمة الاكروسوم مقارنة بمعاملة السيطرة طوال الأوقات المختلفة للتجربة. يمكن الاستنتاج بأن إضافة 0.5 مليمول من Co–Q10 أدى إلى تحسين نوعية السائل المنوي بعد الحفظ بالتجميد لثيران الهولشتاين، وهذا بدوره سيعمل على زيادة معدل الخصوبة للأبقار الملقحة اصطناعيا وبالتالي استدامة نمو الاقتصاد. إن تحسين نوعية السائل المنوي من شأنه أن يسهم في رفع إنتاجية الحيوانات، بما يساعد على تلبية الطلب الاستهلاكي المتزايد، ومواكبة النمو السكاني المتسارع، والتكيف مع التغيرات المناخية التي يشهدها كوكب الأرض في الوقت الراهن.

الكلمات المفتاحية: مضادات الاكسدة، الحفظ بالتجميد، مخفف السائل المنوي، استدامة نمو الاقتصاد.