University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 83-92

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/88t6pg20*

Melatonin, Niacin and Copper Supplementation in Cooling Tris Extender Enhances buffalo bulls semen characteristics

Layth Abdul hussein Chyad , Yassen Taha Abdul-Rahaman

College of Veterinary Medicine, University of Fallujah, Al-Fallujah, Iraq
Y. T. Abdul-Rahaman, College of Veterinary Medicine, University of Fallujah, Iraq, Iraq.
Email: **yassentaha@uofallujah.edu.iq**

Abstract

This study explored the effect of melatonin, niacin and copper supplementation on sperm quality during different cooling periods of Iraqi buffalo bulls. Four adult bulls were used and divided equally into 4 groups. Melatonin (G1), Niacin (G2), Copper (G3) were added to tris extender and compared with the control group (C). Semen was collected for 7 weeks and cryopreserved during the cooling periods (0, 24, 48 and 72 days). The results of the cooling on individual motility show significant (P≤0.05) differences between the groups, with the G1, G2, and G3 outperforming the C in the periods. The percentage increased in the G1 and G2 and lower G3, while individual motility decreased in C. The percentage of live sperm also increased significantly (P≤0.05) in the G1, G2, and G3 during the different cooling periods, and decreased significantly in the C. G1 group exhibited a decreased abnormal sperm percentage when measured against the remaining groups. The results of the sperms plasma membrane integrity showed a significant (P≤0.05) superiority of the G1 during the periods of cooling, over the rest of the G2, G3 and C respectively. No significant differences in the acrosome integrity of sperm in the semen preserved at different cooling periods among the four groups. In conclusion, addition of melatonin, niacin, and copper to the semen diluent of Iraqi buffalo bulls significantly affected semen characteristics during different cooling periods, with melatonin being superior in improving some of these characteristics.

Keywords: Antioxidant, Cooling, Semen, Quality, Iraqi buffalo bulls

I. Introduction

Antioxidants are compounds that can reduce the harmful effects of oxidative stress on sperm during sperm production and storage. Antioxidants can act as scavengers of reactive oxygen species (ROS) produced by lipid peroxidation (LPO), improving the motility and fertilization probability of buffalo sperm (Lone *et al*., 2018).

Buffalo semen has an antioxidant stress protection system that includes both enzymatic and non-enzymatic antioxidants (Turaja *et al*., 2019). However, during cryopreservation, the endogenous defense system is unable to counteract this stress due to the low concentration of these antioxidants in sperm (Ansari *et al*., 2012). Studies have shown that buffalo semen has poor shelf life and cryopreservation properties (Patel *et al*., 2016). The negative changes in buffalo sperm are due to its unique physiological structure and higher concentration of polyunsaturated phospholipids in the plasma membrane (Sansone *et al*., 2000). The plasma membrane of buffalo sperm contains a high proportion of polyunsaturated fatty acids (Silva *et al*., 2020), which makes it more susceptible to oxidative stress during cryopreservation. As a result, buffalo sperm have reduced motility and decreased integrity of the plasma membrane, acrosome, and chromatin. This weakness is mainly due to the low concentration of antioxidants in buffalo semen. These decrease during the cryopreservation process. Abnormal, immature, and dead sperm continuously generate free radicals in the seminal plasma, and the cryopreservation process is associated with a decrease in the concentration of antioxidants in the seminal plasma, which seriously affects the quality of bull semen (Alhelal and Abdulkareem, 2023a; Nsaif and Eidan, 2023) and causes oxidative stress in sperm. The addition of some antioxidants to bull semen has a good effect on improving semen quality after cryopreservation (Alhelal and Abdulkareem, 2023b). This study explores the correlation between Melatonin, Niacin and Copper Supplementation and different cooling periods on semen characteristics of the Iraqi buffalo bulls.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 83-92

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/88t6pg20

II. Materials and Methods

This study was carried out at the Ministry of Agriculture/ Directorate general of Animal Resource/ Department of Artificial Insemination in Abu Ghraib, Iraq. Four Iraqi buffalo bulls (7–8 years old and 500–820 kg weighting) were trained for semen collection using an artificial vagina. One consecutive ejaculate was collected from each bull weekly for successive seven weeks using an artificial vagina. The semen samples were assessed for ejaculate volume, concentration of sperm, and motility percentage.1 ml was taken from each ejaculate to make pooled semen in order to eliminate individual differences between bulls, and then split into 4 equal portions and diluted at 37°C with Tris-based extender supplemented with different groups: G1: Adding fresh semen to a Melatonin (Bulk Supplement.com, USA) (1.1614 g/ 100 ml) containing Tris diluent was involved. G2: Adding fresh semen to a Niacin (Bulk Supplement.com, USA) (24.622 g/ 100 ml) containing Tris diluent was involved. G3: Adding fresh semen to a Copper (Bulk Supplement.com, USA) (0.016 g/ 100 ml) containing Tris diluent was involved. C (Control): This involved adding fresh semen to the Tris diluent only. The semen was diluted to 100 ml at 37°C for the purpose of preserving buffalo bull semen. The semen samples were then placed at 32°C and transferred to a refrigerator (5°C) (Shukla, 2020). Individual motility percentage, live sperm percentage, abnormal sperm percentage, sperms plasma membrane integrity and acrosome integrity of sperm were evaluated after cooling for 0, 24, 48, and 72 hours (Srivastava and Pande, 2017).

The statistical computations were performed using SAS (2018) program based on study on completely randomized deign to study the effect of different factors on the studies characteristics. $Yij = \mu + Ti + eij$ Yij = dependent variable pertaining to the j observation of the i treatment μ = overall mean Ti = effect of the treatment (i- T1, T2 and T3 groups). eij = error term means with significant differences were compared using the Least Significant Differences test (LSD) according to Steel and Torrie (1980).

III. Results and Discussion

Effect of groups on Individual motility (%) of Iraqi buffalo bulls during different cooling periods.

The results of the cooling preservation tests show significant ($P \leq 0.05$) differences between the groups, with the G1 (36.429 ± 1.429, 33.571 ± 0.922, 32.857 ± 1.010, 32.857 ± 1.010)%, G2 (35 ± 1.890, 32.143 ± 1.487, 32.143 ± 1.487, 32.143 ± 1.487)%, and G3 (32.143 ± 1.487, 31.429 ± 0.922, 30 ± 1.543, 30 ± 1.543)% outperforming the C (26.429 ± 0.922, 26.429 ± 0.922, 27.143 ± 1.487, 27.143 ± 1.487)% in the periods (0, 24, 48, 72) hours respectively (Table 1).

The current results revealed that melatonin supplementation to Tris extender prior to cooling resulted in fantastic and protecting functions on semen quality. The 1.1614 g/ 100 ml concentrations of melatonin higher the quality of sperm motility. Melatonin plays a crucial role in protecting sperm from free radical damage. It also binds to its receptors on the mitochondrial membrane, which interact with Calmodulin, stimulating Ca^{+2} influx into sperm cells and the cyclic-Adenosine Monophosphate (cAMP), through which sperm obtain the energy necessary for motility (Ashrafi *et al*., 2013). Melatonin also increases sperm motility by lowering Acid phosphatase levels. Furthermore, melatonin plays a role in increasing energy production by interacting with the electron transport chain within mitochondria and enhancing its activity (Anwar *et al*., 1996; Martin *et al*., 2000).

The results of the current study did not agree with Bahrami *et al*. (2020), who observed a significant decrease in sperm motility in all study groups during the cold storage period during the addition of niacin to the diluent. Niacin is one of the essential vitamins for energy metabolism. It plays a vital role in the citric acid cycle, through fat metabolism in cholesterol synthesis and steroid production, as well as protein and carbohydrate metabolism (Calsamiglia and Rodríguez, 2012). These results indicate that niacin played a protective role for sperm by acting as an antioxidant, reducing lipid peroxidation and preventing membrane damage (Contri *et al*., 2011).

Copper has been identified as playing a vital role in spermatogenesis and male infertility (Sakhaee *et al*., 2012). While its specific function in spermatozoa remains uncertain, it seems to contribute to sperm motility and might also influence pituitary receptors that regulate the release of LH (Yunus *et al*., 2015). Copper functions as an antioxidant, primarily serving as a cofactor for certain enzymes like Cu superoxide dismutase (SOD). It plays a vital role in safeguarding spermatozoa from peroxidative damage caused by reactive oxygen species, protecting their cellular enzymes and structures. The influence of copper levels in

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 83-92

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/88t6pg20*

seminal plasma on sperm parameters, such as sperm motility, has been observed in buffaloes (Tabassomi and Alavi-Shoushtari, 2013).

Table (1) Effect of groups on individual motility (%) of Iraqi buffalo bulls during different cooling periods

Periods / Groups	Cooling periods (hours)			
	0	**24**	**48**	**72**
G1	36.429 ± 1.429* a	33.571 ± 0.922 a	32.857 ± 1.010 a	32.857 ± 1.010 a
G2	35 ± 1.890 a	32.143 ± 1.487 a	32.143 ± 1.487 a	32.143 ± 1.487 a
G3	32.143 ± 1.487 a	31.429 ± 0.922 a	30 ± 1.543 ab	30 ± 1.543 ab
C	26.429 ± 0.922 b	26.429 ± 0.922 b	27.143 ± 1.487 b	27.143 ± 1.487 b
Level of significant	0.0004	0.0007	0.0351	0.0351

* Mean ± Standard Error

NS: Non-significant within the same column show at a significant level (P≤0.05).

a, b: Different letters within the same column show significant differences (p<0.05)

Effect of groups on live sperm percentage (%) of Iraqi buffalo bulls during different cooling periods.

The results of the current study show significant differences (P≤0.05) in the live sperm percentage (%) between the different experimental groups and for different cooling periods, as the G1 (67.857 ± 2.143, 65 ± 1.890, 65 ± 1.890, 65 ± 1.890)%, G2 (62.143 ± 1.010, 60.714 ± 1.304, 60.714 ± 1.304, 60.714 ± 1.304)%, and G3 (63.571 ± 2.103, 61.429 ± 1.798, 61.429 ± 1.798, 60.714 ± 1.304)% respectively, outperformed the C (55.714 ± 2.296, 47.143 ± 2.405, 41.429 ± 0.922, 41.429 ± 0.922)% (Table 2).

The quality of ejaculate is determined by the percentage of live spermatozoa present. Ejaculates containing over 30% dead spermatozoa initially are often considered unsuitable for cooling processes and typically discarded. The live percent of spermatozoa depends on the factors like age, temperature, frequency of semen collections and sexual excitement before collection (Singh *et al*., 2018). The semen is chilled to a temperature of 4°C to 5°C after dilution to slow down spermatozoa metabolism. Gradual cooling is necessary to avoid cold shock, which can impair membrane proteins essential for maintaining structural integrity and ion exchange. Buffalo spermatozoa are particularly sensitive to cryoinjury during the cooling process. However, this damage can be minimized by using suitable diluents and optimizing both the cooling rate (Yeni and Kirikkulak, 2024).

The melatonin-enhancing effect could be attributed to its powerful ability to scavenge nitric oxide (NO) and reactive oxygen species (ROS), which directly and indirectly influence sperm viability by initiating sperm apoptosis (Chaudhary *et al*., 2021).

The current study did not agree with Bahrami *et al*. (2020), as he noted that niacin at 15 and 30 mM concentrations could improve the live sperm percentage during cold storage. Niacin has the potential to enhance sperm viability while significantly reducing reactive oxygen species (ROS). However, sperm response to H2O2-induced acrosome reactions is influenced by the presence of antioxidants, which can inhibit this process. Furthermore, the acrosome reaction has been linked to ROS, with these species playing a role in catalyzing and regulating the reaction (De Lamirande *et al*., 1997).

Some studies have shown that high levels of copper negatively affect the live sperm percentage (Roychoudhury *et al*., 2016). Bank voles exposed to 150 and 600 mg/kg of copper over a 12-week period exhibited reduced viability (Schramm *et al*., 2014). Eghbali *et al*. (2008) demonstrated that the copper content present in buffalo seminal plasma impacts viability, one of the key factors influencing semen fertility. Copper acts as a cofactor in mitochondrial cytochrome c oxidase and is essential for aerobic ATP production in sperm. Its presence is linked to heightened expression and activity of SOD-1, CAT, and GPX4 in sperm, along with reduced lipid peroxidation levels (Ferrer *et al*., 2024).

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 83-92

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/88t6pg20*

The superiority of the first three groups of live sperm after cooling may be due to the difference in exposure to cold shock during cooling. This leads to a change in the distribution of calcium ions between the sperm cell and seminal plasma, resulting in a decrease in the calcium ion content of the seminal plasma and an increase in the concentration of protein-bound calcium in the sperm. Changes in the concentration of positive ions and enzymes in the sperm also occur during cooling, affecting their live sperm (Gitto *et al*., 2001). Evaluating and comparing various techniques for assessing sperm viability may help uncover the relationship between seasonal variations and the viability of sperm in buffalo semen (Ahmad *et al*., 2022).

Table (2) Effect of groups on live sperm percentage (%) of Iraqi buffalo bulls during different cooling periods

Periods / Groups	Cooling periods (hours)			
	0	**24**	**48**	**72**
G1	67.857 ± 2.143* a	65 ± 1.890 a	65 ± 1.890 a	65 ± 1.890 a
G2	62.143 ± 1.010 a	60.714 ± 1.304 a	60.714 ± 1.304 a	60.714 ± 1.304 a
G3	63.571 ± 2.103 a	61.429 ± 1.798 a	61.429 ± 1.798 a	60.714 ± 1.304 a
C	55.714 ± 2.296 b	47.143 ± 2.405 b	41.429 ± 0.922 b	41.429 ± 0.922 b
Level of significant	0.0021	<0.0001	<0.0001	<0.0001

* Mean ± Standard Error

a, b: Different letters within the same column show significant differences ($p<0.05$)

Effect of groups on Abnormal sperm percentage (%) of Iraqi buffalo bulls during different cooling periods.

Table (3) indicates that the percentage of abnormal sperm in the G1, G2, and G3 groups was considerably ($P\geq0.05$) reduced during the initial cooling storage period (16.143 ± 1.370, 18.571 ± 1.571, 19.714 ± 1.375)% in comparison to the C group (26 ± 1.309)%. During the cooling storage times of 24, 48, and 72 hours, the G1 group exhibited a decreased abnormal sperm percentage (18.714 ± 1.584, 18.714 ± 1.584, 18.714 ± 1.584)% when measured against the remaining groups. In contrast, the G2 and G3 groups showed an increase in abnormal sperm percentages (22.857 ± 1.818, 23 ± 1.902, 23 ± 1.902)% and (23.857 ± 1.487, 24.286 ± 1.459, 24.429 ± 1.343)%, respectively, with the C group experiencing the most significant rise (32.143 ± 1.487, 35 ± 1.091, 35 ± 1.091)%.

Table (3) Effect of groups on Abnormal sperm percentage (%) of Iraqi buffalo bulls during different cooling periods

Periods / Groups	Cooling periods (hours)			
	0	**24**	**48**	**72**
G1	16.143 ± 1.370* b	18.714 ± 1.584 c	18.714 ± 1.584 c	18.714 ± 1.584 c
G2	18.571 ± 1.571 b	22.857 ± 1.818 bc	23 ± 1.902 bc	23 ± 1.902 bc
G3	19.714 ± 1.375 b	23.857 ± 1.487 b	24.286 ± 1.459 b	24.429 ± 1.343 b
C	26 ± 1.309 a	32.143 ± 1.487 a	35 ± 1.091 a	35 ± 1.091 a
Level of significant	0.0004	<0.0001	<0.0001	<0.0001

* Mean ± Standard Error

a, b, c: Different letters within the same column show significant differences ($p<0.05$)

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 83-92

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/88t6pg20

The abnormal sperm were affected by several factors, including age, breed, mating season, and the frequency of collection. The causes of deformities are genetic, disease, and environmental (Javed *et al*., 2018; Goncalves *et al*., 2021).

Melatonin plays an important role in reducing sperm abnormalities by lowering the level of acid phosphatase enzyme (Anwar *et al*., 1996). Melatonin mitigates oxidative stress in both fresh and cryopreserved semen, attributable to its capacity to eliminate ROS (Nsaif and Eidan, 2024).

In Bahrami *et al*. (2020) study no significant differences were observed when niacin was added at different concentrations to the semen of stallion on abnormal morphology, but increased significantly at hours 18 and 42 of cold storage. As an antioxidant, niacin did not significantly reduce the percentage of abnormal sperm in our current study, as the percentage of abnormalities increased in the niacin group.

Regarding the effect of copper on sperm abnormalities, Schramm *et al*. (2014) noted that adding 150 and 600 mg/kg Cu for 12 weeks showed low spermatozoa head abnormalities, while higher dose compromised spermatozoa tail in Bank voles. Copper plays a critical role in spermatogenesis and sperm functionality; however, excessive levels of copper, known as hypercuprosis, can be harmful. Highly reactive free cuprous ions (Cu^{+}) interact with hydrogen peroxide (H_2O_2), leading to the formation of hydroxyl radicals through Fenton and Haber-Weiss reactions. Additionally, copper may directly bind to free thiol groups in cysteine residues, resulting in oxidation and the formation of crosslinks between proteins. This interaction can inactivate enzymes or disrupt the function of structural proteins. Elevated dietary intake of copper has been associated with increased sperm abnormalities, heightened ROS production, and enhanced lipid peroxidation (Ferrer *et al*., 2024). Khaki and Araghi (2024) demonstrated that copper exerts a toxic impact on the epithelium of the seminiferous tubules, leading to a rise in abnormal sperm production. In our current study, the addition of copper negatively affected the percentage of sperm abnormalities, as the percentage of abnormalities increased.

Effect of groups on sperms plasma membrane integrity (%) of Iraqi buffalo bulls during different cooling periods.

The results of the statistical analysis (Table 4) showed a significant (P≤0.05) superiority of the G1 in the sperms plasma membrane integrity during the periods of cooling (0, 24, 48, 72) hours (28.571 ± 0.922, 33.286 ± 0.892, 33.286 ± 0.892, 33.571 ± 0.922)% respectively, over the rest of the G2 (22.714 ± 1.209, 25.857 ± 1.204, 26.143 ± 1.033, 27.571 ± 1.172)%, G3 (22.857 ± 1.010, 27.143 ± 1.487, 27.143 ± 1.487, 27.143 ± 1.487)% and C (19.286 ± 0.714, 22.857 ± 1.010, 24.286 ± 0.714, 24.286 ± 0.714)% respectively.

Table (4) Effect of groups on sperms plasma membrane integrity (%) of Iraqi buffalo bulls during different cooling periods

Periods / Groups	Cooling periods (hours)			
	0	**24**	**48**	**72**
G1	28.571 ± 0.922* a	33.286 ± 0.892 a	33.286 ± 0.892 a	33.571 ± 0.922 a
G2	22.714 ± 1.209 b	25.857 ± 1.204 bc	26.143 ± 1.033 b	27.571 ± 1.172 b
G3	22.857 ± 1.010 b	27.143 ± 1.487 b	27.143 ± 1.487 b	27.143 ± 1.487 b
C	19.286 ± 0.714 c	22.857 ± 1.010 c	24.286 ± 0.714 b	24.286 ± 0.714 b
Level of significant	<0.0001	<0.0001	<0.0001	<0.0001

* Mean ± Standard Error

a, b, c: Different letters within the same column show significant differences (p<0.05)

The structure and functionality of sperm are influenced at every stage, potentially resulting in alterations to the integrity of the sperm membrane integrity (Yeni and Kirikkulak, 2024). Cryopreservation causes certain irreversible damage to sperm cells. Key factors contributing to these damages include temperature fluctuations, exposure to reactive oxygen species, lipid peroxidation, changes in the sperm membrane, the toxicity of cryoprotectants, and osmotic stress (Medeiros *et al*., 2002).

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 83-92

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/88t6pg20

The plasma membrane of buffalo bull spermatozoa contains a high concentration of polyunsaturated fatty acids (PUFAs). These PUFAs render the membrane highly susceptible to lipid peroxidation (Shah *et al*., 2017), during semen preservation, oxidative stress (OS) leads to an increase in ROS production, which in turn elevates LPO levels in the spermatozoa membrane (Silvestre *et al*., 2021).

The cooling process lowers the metabolic activity of sperm cells. Insufficient cooling can compromise membrane integrity by causing protein misalignment, interfering with ion channels, generating reactive oxygen species (ROS), and decreasing mitochondrial membrane potential. Typically, seminal plasma is rich in both enzymatic and non-enzymatic antioxidants. However, the ability to counteract oxidative stress is notably weakened when semen is diluted with extenders prior to storage (Inyawilert *et al*., 2024).

The melatonin supplementation at 1.1614 g/ 100 ml might have eliminated the toxic ROS.

Niacin acted as an antioxidant, protecting sperm by reducing lipid peroxidation, which otherwise leads to membrane damage and a loss of membrane function and integrity (Contri *et al*., 2011; Partyka *et al*., 2012). Niacin enhanced the integrity of the plasma membrane, inhibited lipid peroxidation, and increased levels of PUFAs. These findings suggest that Niacin mitigated the formation of free radicals caused by osmotic stress, and cold shock during process. By stabilizing the plasma membrane, it effectively reduced lipid peroxidation of PUFAs, thereby protecting the membrane (Lee *et al*., 2019).

As for the effect of copper on sperm plasma membrane integrity, Schramm *et al*. (2014) noted that administering a higher dose of copper compromised sperm membrane integrity in Bank voles. The addition of copper at a rate of 0.016 g/ 100 ml may have been associated with increased expression and activity of SOD-1, CAT, and GPX4 in sperm and decreased lipid peroxidation (Ferrer *et al*., 2024).

Effect of groups on Acrosome integrity of sperm (%) of Iraqi buffalo bulls during different cooling periods.

The results showed no statistically significant differences in the Acrosome integrity of sperm in the semen preserved at different Cooling periods among the four experimental Groups. The total number ranged from (27.143 ± 1.487- 30.714 ± 0.714)% in the 0-hour period, from (30.714 ± 0.714- 34.286 ± 1.304)% in the 24-hour period, from (30.714 ± 0.714- 34.286 ± 1.304)% in the 48-hour period, and from (30.714 ± 0.714- 34.286 ± 1.304)% in the 72-hour period (Table 5).

Table (5) Effect of groups on Acrosome integrity of sperm (%) of Iraqi buffalo bulls during different cooling periods

Periods / Groups	Cooling periods (hours)			
	0	**24**	**48**	**72**
G1	27.143 ± 1.487*	31.429 ± 1.798	32.143 ± 1.487	32.143 ± 1.487
G2	27.143 ± 1.010	30.714 ± 0.714	30.714 ± 0.714	30.714 ± 0.714
G3	27.143 ± 1.010	30.714 ± 0.714	32.143 ± 1.010	32.857 ± 1.010
C	30.714 ± 0.714	34.286 ± 1.304	34.286 ± 1.304	34.286 ± 1.304
Level of significant	NS	NS	NS	NS

* Mean ± Standard Error

NS: Non-significant within the same column show at a significant level (P≤0.05).

The current study agreed with Nasif (2023) that there were no significant differences in the effect of melatonin hormone on sperm acrosome integrity.

Our study did not align with the findings of Bahrami et al. (2020), who reported that the addition of niacin at concentrations of 20 and 40 mM could equally and significantly reduce the number of 6 hours of cold storage.

The integrity of sperm acrosomes varies depending on the breed, age, diluent used, liquefaction rate, and evaluation procedures (Nitharwal *et al*., 2016). Research has shown that egg yolks can influence sperm acrosome integrity. Due to their significant steroid content, including pregnenolone and progesterone, egg yolks can trigger an acrosomal reaction in sperm cells. This process raises the likelihood of microbial contamination, the presence of drug residues, and hormonally active compounds in both the egg yolk and extended semen (Ritar and Salamon, 1982). Jainudeen and Das (1982) noted that the percentage of cholesterol added to the seminal fluid diluent has a significant effect on sperm acrosome integrity, with a 3-

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 83-92

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/88t6pg20

5% cholesterol concentration being better than other concentrations. The percentage of Acrosome integrity of sperm in fresh buffalo semen ranges from 5-18%. Acrosome detachment occurs due to low ATP levels and intracellular protein loss. Acrosin and hyaluronidase enzymes are present in small quantities in the acrosome of buffalo bulls (Pant *et al*., 2002).

The lack of significant differences in the effect of the four groups on sperm acrosome integrity during different cooling periods may be due to the exposure of sperm to cold shock during cooling, which in turn leads to changes in the distribution of calcium ions between the sperm cell and seminal plasma, which leads to a decrease in the contents of seminal plasma of calcium ions and an increase in the concentration of protein-bound calcium in the sperm, as well as changes in the concentrations of positive ions and enzymes in the sperm during cooling, and thus a decrease in the percentage of sperm acrosome integrity (Gitto *et al*., 2001).

IV. Conclusions

The incorporation of melatonin (1.1614 g/ 100 ml), niacin (24.622 g/ 100 ml), and copper (0.016 g/ 100 ml) into the semen diluent for Iraqi buffalo bulls had a significant impact on semen quality across various cooling durations (0, 24, 48, 72) hours. Among these additives, melatonin proved to be particularly effective in enhancing certain semen characteristics.

V. References

Ahmad, E.; Shakeel, M.; Naseer, Z.; Ahmed, A.; Nawaz, M. and Akhtar, M.S. (2022). Cryopreservation of Nili-Ravi buffalo bull semen: yearly post-thaw analyses to depict climatic effects. Turkish Journal of Veterinary and Animal Sciences, 46(6): 803- 808. https://doi.org/10.55730/1300-0128.4256

Alhelal, A.M. and Abdulkareem, T.A. (2023a). Ameliorating post-thawed semen of buffalo bulls using a milk-based extender supplemented with resveratrol. Iraqi Journal of Agricultural Sciences, 55(Special Issue): 186-194. https://doi.org/10.36103/ijas.v55iSpecial.1897

Alhelal, A.M. and Abdulkareem, T.A. (2023b). Effect of adding resveratrol to soybean-lecithin extender on some semen attributes of buffalo bulls. Iraqi Journal of Agricultural Sciences, 54(4): 1074-1083.https://doi.org/10.36103/ijas.v54i4.1797

Ansari, M.S.; Rakha, B.A.; Andrabi, S.M.H.; Ullah, N.; Iqbal, R.; Holt, W.V. and Akhter, S. (2012). Glutathione-supplemented tris-citric acid extender improves the post-thaw quality and in vivo fertility of buffalo (*Bubalus bubalis*) bull spermatozoa. Reproductive Biology, 12(3): 271–276. https://doi.org/10.1016/j.repbio.2012.10.001

Anwar, M.M.; Megahed, G.A.; El-Deeb, T.S. and Shehata, H.S. (1996). The effect of melatonin on the bull liquid semen and enzymatic release in seminal plasma. Assiut Veterinary Medical Journal, 35(69): 42- 62.

Ashrafi, I.; Kohram, H. and Ardabili, F.F. (2013). Antioxidative effects of melatonin on kinetics, microscopic and oxidative parameters of cryopreserved bull spermatozoa. Animal Reproduction Sciences, 139(1-4): 25-30. https://doi.org/10.1016/j.anireprosci.2013.03.016

Bahrami, A.; Divar, M.R.; Azari, M. and Kafi, M. (2020). Nicotinic Acid (Niacin) Supplementation in Cooling and Freezing Extenders Enhances Stallion Semen Characteristics. Journal of Equine Veterinary Science, 94(103236): 1- 9. https://doi.org/10.1016/j.jevs.2020.103236

Calsamiglia, S. and Rodríguez, M. (2012). Optimum vitamin nutrition in dairy cattle. In: Optimum vitamin nutrition in the production of quality animal foods. Barroeta, A.C.; Baucells, M.D.; Blanco Pérez, A.; Calsamiglia, S.; Casals, R.; Cepero Briz, R.; Davin, R.; Gonzalez, G.; Hernandez, J.M.; Isabel, B.; Lopez Bote, C.; Rey, I.A.; Rodriguez, M.; Sanz, J.; Soto-Salanova, M.F. and Weber, G. Chapter 7, DSM Nutritional Products Limited, UK. P. 359.

Chaudhary, S.C.; Aeksiri, N.; Wanangkarn, A.; Liao, Y.J. and Inyawilert, W. (2021). Effects of melatonin on cryopreserved semen parameters and apoptosis of thai swamp buffalo bull (bubalus bubalis) in different thawing conditions. Advances in Animal and Vetereinary Sciences, 9(2): 238-245. http://dx.doi.org/10.17582/journal.aavs/2021/9.2.238.245

Contri, A.; De Amicis, I.; Molinari, A.; Faustini, M.; Gramenzi, A.; Robbe, D. and Carluccio, A. (2011). Effect of dietary antioxidant supplementation on fresh semen quality in stallion. Theriogenology, 75(7): 1319-1326. http://dx.doi.org/10.1016/j.theriogenology.2010.12.003

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 83-92

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/88t6pg20

De Lamirande, E.; Tsai, C.; Harakat, A. and Gagnon, C. (1997). Involvement of reactive oxygen species in human sperm arcosome reaction induced by A23187, lysophosphatidylcho- line, and biological fluid ultrafiltrates. Journal of Andrology, 19(5):585- 594.

Eghbali, M.; Alavi-Shoushtari, S.M. and Asri Rezaii, S. (2008). Effects of copper and superoxide dismutase content of seminal plasma on Buffalo semen characteristics. Pakistan Journal of Biological Sciences, 11(15): 1964- 1968. http://dx.doi.org/10.3923/pjbs.2008.1964.1968

Ferrer, M.; Palomares, R. and Maldonado-Estrada, J. (2024). Role of trace minerals in bull reproductive physiology and semen quality. Clinical Theriogenology, 16 (10351): 1- 7. https://doi.org/10.58292/CT.v16.10351

Gitto, E.; Tan, D.X.; Reiter, R.J.; Karbownik, M.; Manchester, L.C.; Cuzzocrea, S.; Fulia, F. and Barberi, I. (2001). Individual and synergistic antioxidative actions of melatonin: Studies with vitamin E, vitamin C, glutathione and desferrrioxamine (desferoxamine) in rat liver homogenates. The Journal of Pharmacy Pharmacology, 53(10): 1393-1401. https://doi.org/10.1211/0022357011777747

Goncalves, A.A.; Garcia, A.R.; Rolim Filho, S.T.; da Silva, J.A.R.; de Melo, D.N.; Guimarães, T.C.; Tavares, H.R., Silva, T.V.G.; de Souza, E.B.; Santos, S.D.D. and Ohashi, O.M. (2021). Scrotal thermoregulation and sequential sperm abnormalities in buffalo bulls (Bubalus bubalis) under short-term heat stress. Journal of Thermal Biology, 96: 102842. https://doi.org/10.1016/j.jtherbio.2021.102842

Inyawilert, W.; Liao, Y.; Ndi, O.N.; Pradithera, K.; Saengtun, A.; Saengwong, S.; Intawicha, P.; Wuthijaree, K.; Hanthongkul, V.; Kamdee, K.; Khieokhajonkhet, A.; Amporn, C.; Attapol Tiantong, A. and Lumsangkul, C. (2024). The optimal combination of cooling and equilibration durations, along with the addition of melatonin, gamma-oryzanol, and canthaxanthin, for improving swamp buffalo semen cryopreservation quality. Veterinary World, 17(12): 2950–2956. https://doi.org/10.14202/vetworld.2024.2950-2956

Jainudeen, M.R. and Das, S. (1982). Effect of level of glycerol, rates of freezing and thawing on the survival of buffalo spermatozoa in straws. In: Jainudeen, M.R. and Omar, A.R. (eds), Proceedings of Asian-Australian Anim. Sci. Cong, Serdang, Malaysia. Penerbit University, Kuala Lumpur, Malaysia, PP. 409–411.

Javed, M.T.; Ahmad, N.; Saleem, G.; Sadia, H.; Manzoor, F. and Dilbar, G.H. (2018). Effect of age and season on semen characteristics of buffalo bull- A review. Journal of Innovative Sciences, 4(1): 65- 82.https://doi.org/10.13140/RG.2.2.13510.09287

Khaki, A. and Araghi, A. (2024). Comparison of sperm quality, blood and seminal plasma element and enzyme levels in sub-fertile and healthy bull. Caspian Journal of Veterinary Sciences, 1(2): 135-144.

Lee, S.H.; Kim, Y.J.; Kang, B.H. and Park, C.K. (2019). Effect of nicotinic acid on the plasma membrane function and polyunsaturated fatty acids composition during cryopreservation in boar sperm. Reproduction in Domestic Animals, 54: 1251- 1257. https://doi.org/10.1111/rda.13508

Lone, S.; Prasad, J.; Ghosh, S.; Das, G.; Balamurugan, B. and Verma, M. (2018). Study on correlation of sperm quality y parameters with antioxidant and oxidant status of buffalo bull semen during various stages of cryopreservation. Andrologia, 50(4): e12970. https://doi.org/10.1111/and.12970

Martin, M.; Macías, M.; Escames, G.; León, J. and Acuña-Castroviejo, D. (2000). Melatonin but not vitamins C and E maintains glutathione homeostasis in t-butyl hydroperoxide-induced mitochondrial oxidative stress. FASEB Journal, 14(12): 1677- 1679. https://doi.org/10.1096/fj.99-0865fje

Medeiros, C.M.O.; Forell, F.; Oliveira, A.T.D. and Rodrigues, J.L. (2002). Current status of sperm cryopreservation: why isn't it better? Theriogenology, 57(1): 327-344.https://doi.org/10.1016/S0093-691X(01)00674-4

Nasif, Z.M. (2023). Effect of melatonin implantation on fresh and cryopreserved semen quality of Iraqi buffalo bulls. MSc. Thesis Agricultural Sciences- Animal Production/ Reproductive Physiology. College of Agricultural Engineering Science, University of Baghdad, Iraqi.

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 83-92

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/88t6pg20

Nitharwal, K.; Singh, P.; bhakat, M.; Achaurasia, A.; Abdullah, M. and Prabha, C. (2016). Effect of season on semen quality parameters in Murrah buffalo. Indian Journal of Animal Sciences, 87 (1): 64-69. https://doi.org/10.56093/ijans.v87i1.66866

Nsaif, Z. M. and Eidan, S. M. (2023). Effect of melatonin implantation on sexual behavior and some of semen quality of Iraqi Buffalo bulls. IOP Conference Series: Earth and Environmental Science, 1262 (2023): 1- 7. https://doi.org/10.1088/1755-1315/1262/7/072013

Nsaif, Z.M. and Eidan, S.M. (2024). Fresh and cryopreserved semen characteristics of buffalo bulls as influenced by melatonin implantation. Iraqi Journal of Agricultural Sciences, 55(2):644- 651. https://doi.org/10.36103/6erkrp95

Pant, H.C.; Barot, L.R.; Kasiraj, R.; Misra, A.K. and Prabhakar, J.H. (2002). Acrosomal integrity of buffalo spermatozoa and its relation to fertility. The Indian Journal of Animal Reproduction, 23(2): 110-112. https://acspublisher.com/journals/index.php/ijar/article/view/5940

Partyka, A.; Lukaszewicz, E. and Niżański, W. (2012). Lipid peroxidation and antioxidant enzymes activity in avian semen. Animal Reproduction Science, 134(3):184-190. https://doi.org/10.1016/j.anireprosci.2012.07.007

Patel, H.A.; Siddiquee, G.M.; Chaudhari, D.V. and Suthar, V.S. (2016). Effect of different antioxidant additives in semen diluent on cryopreservability (-196 °C) of buffalo semen. Veterinary World, 9(3): 299- 303. https://doi.org/10.14202/vetworld.2016.299-303

Ritar, A.J. and Salamon, S. (1982). Effects of seminal plasma and of its removal and of egg yolk in the diluent on the survival of fresh and frozen–thawed spermatozoa of the angora goat. Australian Journal of Biological Sciences,35(3):305–312.

Roychoudhury, S.; Nath, S.; Massanyi, P.; Stawarz, R.; Kacaniova, M. and Kolesarova, A. (2016). Copper-Induced changes in reproductive functions: in vivo and in vitro effects. Physiological Research, 65(1): 11- 22. https://doi.org/10.33549/physiolres.933063

Sakhaeec, E.; Emadi, L.; Kheirandish, R.; Azari, O.; Abshenas, J. and Amiri, E. (2012). Evaluation of epididymal sperm quality, and histopathological assessment of male reproductive organ, following experimentally induced copper poisoning in male rats. Andrologia, 44: 110-116. https://doi.org/10.1111/j.1439-0272.2010.01147.x

Sansone, G.; Nastri, M.J.F. and Fabbrocini, A. (2000). Storage of buffalo (*Bubalus bubalis*) semen. Animal Reproduction Science, 62(1-3): 55- 76. https://doi.org/10.1016/S0378-4320(00)00154-8

SAS. (2018). Statistical analysis system. User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C., USA.

Schramm, A.M.; Kruczek, M. and Kapusta, J. (2014). Effect of copper exposure on reproductive ability in the bank vole (Myodes glareolus). Ecotoxicology, 23(8): 1546-1554. https://doi.org/10.1007/s10646-014-1295-6

Shah, S.A.H.; Andrabi, S.M.H. and Qureshi, I.Z. (2017). Freezability of water buffalo bull (Bubalus bubalis) spermatozoa is improved with the addition of curcumin (diferuloylmethane) in the semen extender. Andrologia, 49(8): e12713. https://doi.org/10.1111/and.12713

Shukla, M.K. (2020). Cryopreservation of Semen. In: Applied Veterinary Andrology and Frozen Semen Technology. Chapter 8. New India Publishing Agency Pitam Pura, New Delhi-110 088. PP. 77- 80.https://doi.org/10.59317/9789389907445

Silva, L.K.X.; Lourenço, J. D.; da Silva, A.O.A.; de Sousa, J.S.; Silva, A.G.M.; Reis, A.N.D.; Miranda, M.D.S.; Santos, S.D.D.; Ohashi, O.M.;Martorano, L.G.; da Rocha, G.N.; Faturi, C.; de Morais, E.; Mares, E.K.L. and Garcia, A.R. (2020). Increased quality of in natura and cryopreserved semen of water buffaloes supplemented with saturated and unsaturated fatty acids from the palm oil industry. Animal Reproduction, 17(4): 1-17. http://dx.doi.org/10.1590/1984-3143-AR2020-0522

Silvestre, M.A.; Yániz, J.L.; Peña, F.J.; Santolaria, P. and Castelló-Ruiz, M. (2021). Role of Antioxidants in Cooled Liquid Storage of Mammal Spermatozoa. Antioxidants, 10(7): 1096. https://doi.org/10.3390/antiox10071096

Singh, M.; Rajoriya, J.S.; Kumar, A.; Ghosh, S.K. and Prasad, J.K. (2018). Cryopreservation of Buffalo (Bubalus Bubalis) semen: Current status and future prospective. Buffalo Bulletin, 37 (2): 109-128. https://kuojs.lib.ku.ac.th/index.php/BufBu/article/view/42

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 83-92

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/88t6pg20

Srivastava, N. and Pande, M. (2017). Semen Analysis: An Overview Protocols in Semen Biology (Comparing Assays) Springer Nature Singapore Pte Ltd.https://doi.org/10.1007/978-981-10-5200-2

Steel, R. G. D. and Torrie, J. H. (1980). Principles and procedures of statistics. 2nd (ed.,) McGraw Hill, New York.

Tabassomi, M. and Alavi-Shoushtari, S. M. (2013). Effects of in vitro copper sulphate supplementation on the ejaculated sperm characteristics in water buffaloes (Bubalus bubalis). Veterinary Research Forum, 4 (1): 31- 36.

Turaja, K.I.B.; Vega, R.S.A.; Saludes, T.A.; Tandang, A.G.; Bautista, J.A. N.; Salces, A.J. and Rebancos, C. M. (2019). Influence and total antioxidant capacity of non-enzymatic antioxidants on the quality and integrity of extended and cryopreserved semen of murrah buffalo (*Bubalus bubalis*). Philippine Journal of Science, 148(4): 619- 626.

Yeni, D. and Kirikkulak, M. (2024). Cryopreservation of buffalo semen. In: Cryopreservation of semen for farm animals. Tasdemir, U. Chapter 3. Livre De Lyon, Lyon, France, PP. 39- 56. https://doi.org/10.5281/zenodo.13902996

Yunus, E.U.; Mustafa, S.; Mustafa, T. and Baki, H. (2015). Determination of lead, copper and iron in cosmetics, water, soil and food using polyhydroxybutyrate-B-polydimethyl siloxane preconcentration and flame atomic absorption spectroscopy. Analytical Letters, 48(7): 1163-1179.https://doi.org/10.1080/00032719.2014.971365