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EFFECT OF SEPHADEX SPERM FILTRATION AND ANTIOXIDANTS SUPPLEMENTATION ON POST THAW SEMEN QUALITY OF HOLSTEIN BULLS

Hamza Yousef Ali[1] **Sajida Mahdi Eidan**[2]
Hamzayausif120@yahoo.com sajeda.mahdi@coagri.uobaghdad.edu.iq
Omar Adel Mohammed[1]
omaradel19812007@yahoo.com

ABSTRACT

This study aimed to evaluate the efficacy of semen filtration using two different grades of Sephadex in enhancing the quality of low-grade ejaculates from Holstein bulls, in combination with antioxidant supplementation. Semen samples were collected from eight Holstein bulls over a period of eight weeks and subsequently divided into four experimental groups. The first group included SG5, SG6, and SG7, in which low-quality spermatozoa were filtered using Sephadex G-15. The second group consisted of SG8, SG9, and SG10, where low-quality spermatozoa were filtered using Sephadex G-25. A third group (SG1), comprising unfiltered low-quality semen, served as the negative control. Finally, a group of high-quality semen samples (SG2) was designated as the positive control. Groups SG3, SG6, and SG9 were supplemented with a combination of two amino acids (20 mM glycine and 1 mM glutamine), whereas groups SG4, SG7, and SG10 received a supplementation of 0.2 mM vitamin E and 0.8 mg/mL omega-3 fatty acids. Groups SG5 and SG8, which underwent filtration without any supplementation, served as control subgroups. The results demonstrated a significant improvement ($P \leq 0.01$) in sperm motility, viability, normal morphology, acrosome integrity, and plasma membrane integrity in the groups filtered through Sephadex (SG5, SG6, SG7, SG8, SG9, and SG10) following both cooling and cryopreservation. Moreover, groups SG4, SG7, and SG10 showed a significant improvement ($P \leq 0.01$) in sperm motility, viability, and plasma membrane integrity compared to the control groups SG8 and SG1. It can be concluded that sperm filtration is an effective method for reducing dead and abnormal sperm, as well as cellular debris, in low-quality ejaculates of Holstein bulls.

Keywords: bulls, antioxidants, sephadex, cryopreservation, sperm filtration.

INTRODUCTION

Globally, livestock production relies almost entirely on artificial insemination using cryopreserved semen due to its efficiency and the associated economic benefits

[1] College of Agricultural, University of Al-Anbar, Al-Anbar, Iraq.
2 College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.
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[40]. However, cryopreservation of spermatozoa reduces their fertilization capacity and viability. Therefore, to maintain high fertility rates, a higher concentration of spermatozoa is required for artificial insemination compared to the use of fresh semen [31]. During the cryopreservation process, spermatozoa are subjected to physical and chemical damage, including disruptions to the plasma membrane, as well as structural and molecular impairments, which collectively lead to reduced sperm viability and motility. These alterations contribute to oxidative stress [32]. Oxidative stress primarily arises from the excessive production of reactive oxygen species (ROS), which results from an imbalance in the redox state within the seminal plasma [1]. This imbalance disrupts spermatozoa's cellular homeostasis, damaging critical cellular components such as proteins and nucleic acids, including DNA and RNA. Consequently, this oxidative damage impairs sperm motility, acrosomal reaction, and fertilization capacity [34]. Common strategies to improve the quality of cryopreserved semen for artificial insemination include the addition of antioxidants or cryoprotective agents [6], as well as employing alternative cryopreservation techniques such as vitrification or controlled-rate freezing [45].

Seminal plasma naturally contains various substances and factors with antioxidant activity, including enzymatic antioxidants such as superoxide dismutase (SOD), which exhibits antioxidant activity by catalyzing the dismutation of two ROS molecules [16], glutathione peroxidase (GPx), and glutathione reductase (GR), as well as non-enzymatic antioxidants such as methionine and vitamins E and C [6]. However, the endogenous levels of these antioxidant substances are insufficient to counteract the effects of oxidative stress that occur during cryopreservation [47]. Therefore, reducing the concentration of reactive oxygen species (ROS) is essential for preserving sperm motility and viability [48]. One compound with notable antioxidant properties is melatonin, which helps maintain sperm integrity and function [27, 29, 33, 35, 44]. Additionally, vitamins play a significant role in mitigating oxidative stress [37]. For example, ascorbic acid (vitamin C) reduces Fe^{3+} to Fe^{2+}, leading to subsequent reactions with oxygen and hydrogen peroxide [9].

Vitamin E is a lipophilic compound that protects the fatty acids in sperm membranes from oxidative damage [3]. It reacts with free radicals approximately 1,000 times faster than polyunsaturated fatty acids (PUFAs), halting lipid peroxidation [13]. In the absence of vitamin E, PUFAs undergo oxidation readily, leading to the formation of hydrogen peroxide and additional free radicals [20]. Including vitamin E in semen extenders for rams has been shown to preserve mitochondrial function and plasma membrane integrity, thereby enhancing semen quality post-thaw [41]. Moreover, the addition of 1 mg/mL of vitamin E to bull semen extenders has been associated with improved sperm motility and viability following cryopreservation for 96 hours [36]. Numerous studies have confirmed that specific amino acids are critical antioxidants, including heptaurine, glutamine, cysteine, taurine, histidine, proline, and glycine. These compounds have been shown to reduce DNA damage in ram spermatozoa. For instance, the addition of 1, 2, and 4 mM of ergothioneine has been demonstrated to decrease DNA fragmentation in sperm following cryopreservation, primarily by scavenging free radicals and mitigating oxidative stress [14, 46].

Another strategy to improve the quality of cryopreserved semen for artificial insemination involves sperm filtration using conventional techniques such as density gradient centrifugation [28], glass wool filtration [18, 39], swim-up [11, 18], or magnetic-activated cell sorting (MACS; 10). These methods can be applied b efore or after cryopreservation to enhance sperm viability and fertilizing capacity by selectively removing dead and immotile spermatozoa under minimal stress conditions [38]. Sephadex columns represent a cost-effective and straightforward method for sperm filtration by retaining immotile spermatozoa and preventing their passage through the column while allowing progressively motile spermatozoa to pass through [5, 19]. Based on this premise, the present study aimed to evaluate the effectiveness of two types of Sephadex in combination with the addition of a mixture of glutamine, glycine, vitamin E, and omega-3 fatty acids to a Tris-based extender for improving sperm viability during cryopreservation.

MATERIALS AND METHODS

Experimental animals and design

This study was carried out at the Department of Artificial Insemination pertaining to the Directorate of Animal Resources, Ministry of Agriculture, Abu-Ghraib, Baghdad, during the period from January 2023 to May 2023 using 8 Holstein bulls. Both poor fresh semen n=4 bulls and good fresh semen quality n=4 bulls were collected via artificial vagina at two ejaculates\ bull /week semen with less than 50% motility is considered low quality semen, while semen higher than 50% is considered good quality semen . The groups was filtered with Sephadex G15 are SG5, SG6 and SG7 and the groups filtered with Sephadex G25 are SG8, SG9 and SG10. A group left unfiltered considered as a negative control group SG1, A good quality bulls as a positive control group SG2. The groups SG3, SG6 and SG9 was supplemented with a mixture of two amino acids 20 mM glycine + 1 mM glutamine, while groups SG4, SG7 and SG10 was supplemented with a mixture of 0.2 mM vitamin E + 0.8 mg/ml omega3. The groups SG5 and SG8 were left without supplementation and regarded as a control groups.

Semen parameters

Sperm's cell individual motility percentage evaluated according to the method described by Srivastava and Pande [43]. Live sperm percentage was estimated according to the methods described by Hancock [17]. Abnormal sperm percentage was determined according to the methods described by Forouzanfar *et al*. [16] using eosin nigrosine staining technique with similar slide for live sperm estimation, sperm plasma membrane [22] and acrosome integrity [15] sephadex filtration was done according to the method described by Ahmad *et al* [2]. DNA damage and malondialdehyde concentrations according to [43].

Statistical analysis

Statistical analysis conducted using SAS program based on a design completely randomized design (CRD) to study the effect of different factors on semen characteristics. To compare means, we used Duncan's multiple range test. The statistical models for comparison among groups was as follows:

$Y_{ij} = \mu + T_i + e_{ij}$

where Y_{ij} = dependent variable pertaining to the j observation of the i treatment

μ = overall mean

Ti = effect of i treatment

eij = error term

RESULTS AND DISCUSSION

Post-cooling results of sperm motility percentage showed that group SG2 was significantly (P≤0.01) higher than other groups, while group SG1 was the lowest one (Table 1). The groups Sg5-SG10 recorded significant increase (P≤0.01) in sperm motility as compared to SG1 and SG3 groups, and group SG4 had higher (P≤0.01) sperm motility than SG1 (Table 1). Higher (P≤0.01) live sperm percentage was observed in group SG2, while SG1 recorded the lowest group (Table 1). The groups SG5-SG10 exhibited a significant increase (P≤0.01) compared to SG1 and SG3 groups, and SG4 recorded a significant increase (P≤0.01) compared with SG1 group in live sperm percentage (Table 1). Sperm normal morphology percentage revealed that SG1 group was the lowest, and groups SG5 - SG10 higher (P≤0.01) than SG1 and SG3 groups (Table 1). Moreover, group SG4 recorded higher (P≤0.01) sperm normal morphology than the SG1 group, and the group SG2 was the highest percentage compared to all groups (Table 1). Morphological assessment is important semen parameter due to its relation to fertility rate in cattle [26] pigs [46] and horses [23]. Abnormal sperm decreases fertility rate by affecting the sperm's fertilizing ability and motility [25].

`Sperm acrosome integrity was higher (P≤0.01) in groups SG5-SG10 compared with the SG1 and SG3 groups. A significant (P≤0.01) increase was observed in group SG10 compared with SG8 and SG5 groups (Table 1). Sperm plasma membrane integrity was greater in SG5 – SG 10 compared with the SG1 and SG3 groups, and the SG4 group recorded higher plasma membrane integrity than the SG1 group (Table 1).

Table 1: Effect of Sephadex filtration technique and adding of antioxidants on post-cooling semen quality of Holstein bulls (means ±SE).

Group	Sperm motility (%)	Live sperm (%)	Sperm normal morphology (%)	Acrosome integrity (%)	Plasma membrane integrity (%)
SG1	[e] 29.37±1.13	[d] 63.29±1.32	[e] 83.51±0.48	[d] 69.12±1.37	[d] 64.28±1.53
SG2	[a] 52.85±1.48	[a] 86.03±0.73	[a] 96.45±0.36	[a] 92.97±0.83	[a] 89.65±0.65
SG3	[e] 29.00±1.00	[d] 65.21±2.60	[de] 84.96±0.92	[d] 71.21±1.37	[cd] 67.26±1.34
SG4	[d] 35.00±1.82	[c] 69.30±1.50	[cd] 86.28±0.47	[c] 76.89±1.10	[bc] 72.45±1.22
SG5	[cd] 39.28±2.29	[bc] 70.36±1.18	[cd] 86.54±0.43	[bc] 78.92±1.28	[b] 73.20±0.83
SG6	[cd] 38.57±2.60	[bc] 71.79±2.21	[bc] 86.95±0.56	[bc] 79.80±1.39	[b] 74.37±0.67
SG7	[bc] 40.71±2.54	[b] 74.45±2.26	[bc] 87.45±0.43	[bc] 81.36±1.00	[b] 76.59±0.62
SG8	[bc] 40.00±1.54	[bc] 70.37±3.75	[bc] 88.06±1.16	[c] 76.59±1.48	[bc] 72.02±1.59
SG9	[cd] 38.57±2.10	[bc] 71.82±1.92	[bc] 88.20±0.72	[bc] 78.85±1.01	[b] 73.80±2.65
SG10	[b] 44.28±1.70	[bc] 73.53±2.55	[b] 88.74±0.66	[b] 83.06±1.18	[b] 76.29±2.77
Level of significance	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01

SG1= poor semen quality (Tris); SG2= good semen quality (Tris); SG3= SG1+(glutamine-glycine) , SG4= SG1+ vit.E+Omega3; SG5 =Sephadex G15; SG6= SG5+ glutamine-glycine ; SG7=SG5+ vit.E-Omega3; SG8=Sephadex G25 ; SG9= SG8+ glutamine +glycine ; SG10= SG8+ vit.E+ omega3.

Post-cryopreservative sperm motility showed that groups SG5-SG10 were significantly higher (P≤0.01) than those of groups SG1 and SG3. The groups SG7 and SG10 were significantly higher than SG5 and SG8. Further, SG4 group exhibited greater (P≤0.01) sperm motility percentage than those of SG1 and SG3 groups (Table 2). The live sperm percentage were higher (P≤0.01) in groups SG5 – SG10 than those of SG1 and SG3. The groups SG10 and SG7 exhibited greater (P≤0.01) live sperm percentage than SG8 and SG5 (Table 2). The live sperm percentage of group SG4 was significantly (P≤0.01) higher than those of SG1, non-significant difference found between the SG1 and SG3 (Table2). The percentage of sperm with normal morphology was significantly higher (P ≤ 0.01) in groups SG5 through SG10 compared to group SG1 (Table 2). Notably, the SG10 group demonstrated a significantly greater (P ≤ 0.01) proportion of normally shaped sperm compared to the SG8 group. Furthermore, the normal morphology percentage in group SG4 was significantly higher (P ≤ 0.01) than in both SG1 and SG3. However, no significant difference was observed between SG1 and SG3 in this parameter (Table 2). A significantly higher acrosome integrity percentage (P ≤ 0.01) was observed in group SG4 compared to SG1, whereas no significant difference was detected between groups SG1 and SG3 in this parameter (Table 2). Additionally, groups SG5 through SG10 exhibited significantly higher (P ≤ 0.01) acrosome integrity percentages relative to SG1. Among these, group SG7 showed a significantly greater (P ≤ 0.01) percentage than SG5. Moreover, group SG10 demonstrated a significantly higher (P ≤ 0.01) acrosome integrity percentage compared to SG8 (Table 2). No significant differences were observed in plasma membrane integrity percentage between groups SG1 and SG3 (Table 2). However, group SG4 exhibited a significantly higher percentage (P ≤ 0.01) compared to SG1. Groups SG5 through SG10 showed significantly greater (P≤ 0.01) plasma membrane integrity percentages than both SG1 and SG3. Furthermore, groups SG7 and SG10 demonstrated significantly higher (P≤ 0.01) percentages than SG5 and SG8 (Table 2).

Table 2: Effect of Sephadex filtration technique and adding of antioxidants on post-cryopreserved semen quality of Holstein bulls (means ±SE).

Group	Sperm motility (%)	Live sperm (%)	Sperm normal morphology (%)	Acrosome integrity (%)	Plasma membrane integrity(%)
SG1	[f] 20.62±1.75	[e] 50.10±1.20	[d] 73.75±0.55	[e] 57.85±0.86	[e] 50.89±1.43
SG2	[a] 47.50±0.94	[a] 78.62±0.85	[a] 90.83±0.53	[a] 85.21±0.71	[a] 80.84±1.07
SG3	[f] 22.50±1.11	[e] 53.03±2.06	[d] 76.03±2.46	[e] 58.10±2.98	[e] 53.66±1.68
SG4	[e] 30.62±1.75	[d] 61.71±1.68	[c] 80.29±1.14	[d] 69.02±1.46	[cd] 65.19±1.57
SG5	[de] 33.57±2.60	[d] 61.32±1.13	[c] 80.04±0.96	[d] 68.10±1.05	[d] 63.19±1.25
SG6	[cd] 35.00±2.88	[cd] 62.60±3.33	[bc] 81.12±0.51	[cd] 69.53±1.39	[cd] 64.64±0.92
SG7	[bc] 38.57±2.60	[bc] 66. 48±2.23	[bc] 81.86±0.57	[bc] 72.57±1.27	[bc] 68.34±0.85
SG8	[d] 34.16±2.00	[d] 61.81±2.83	[c] 79.15±0.70	[d] 67.96±1.12	[cd] 64.06±1.00
SG9	[cd] 35.00±1.82	[bcd] 64.07±2.79	[c] 80.51±0.68	[cd] 71.54±1.02	[bcd] 67.19±0.66
SG10	[b] 40.83±1.53	[b] 68.15±2.73	[b] 83.39±0.52	[b] 76.54±1.04	[b] 71.23±0.89
Significant	P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01

SG1= poor semen quality (Tris); SG2= good semen quality (Tris) ; SG3= SG1+(glutamine-glycine) , SG4= SG1+ vit.E+Omega3; SG5 =Sephadex G15; SG6= SG5+ glutamine-glycine ; SG7=SG5+ vit.E-Omega3; SG8=Sephadex G25 ; SG9= SG8+ glutamine +glycine ; SG10= SG8+ vit.E+ omega3.

DNA integrity, malondialdehyde and total antioxidants concentrations

The results indicated that group SG2 had the highest percentage of DNA-intact sperm, whereas group SG1 exhibited the lowest (Figure 1). Groups SG5 through SG10 demonstrated significantly higher percentages of DNA-intact sperm ($P \leq 0.05$) compared to groups SG1 and SG3 (Figure 1). Malondialdehyde (MDA) concentrations were significantly lower ($P \leq 0.05$) in groups SG5 through SG10 compared to SG1 (Figure 2). In contrast, the total antioxidant concentrations showed no significant differences between filtered and unfiltered semen groups (Figure 3).

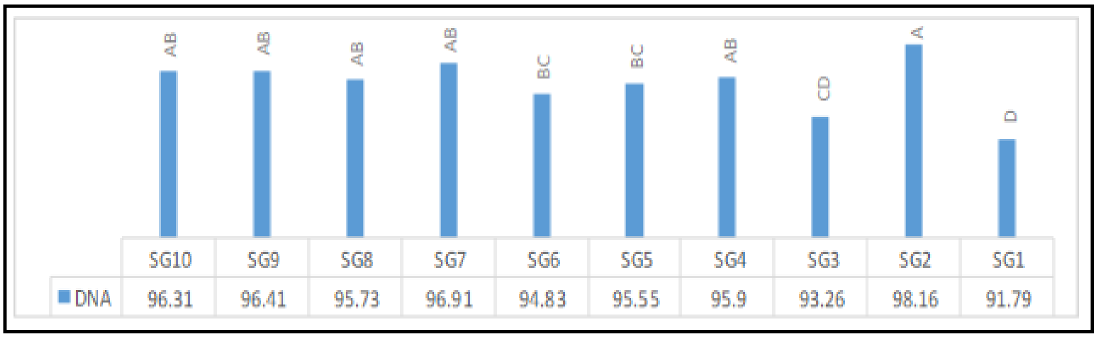

Figure 1: Effect of Sephadex filtration technique and addition of antioxidants on the sperm DNA integrity of Holstein bulls.

SG1= poor semen quality (Tris); SG2= good semen quality (Tris); SG3= SG1+(glutamine-glycine) , SG4= SG1+ vit.E+Omega3; SG5 =Sephadex G15; SG6= SG5+ glutamine-glycine ; SG7=SG5+ vit.E-Omega3; SG8=Sephadex G25 ; SG9= SG8+ glutamine +glycine ; SG10= SG8+ vit.E+ omega3.

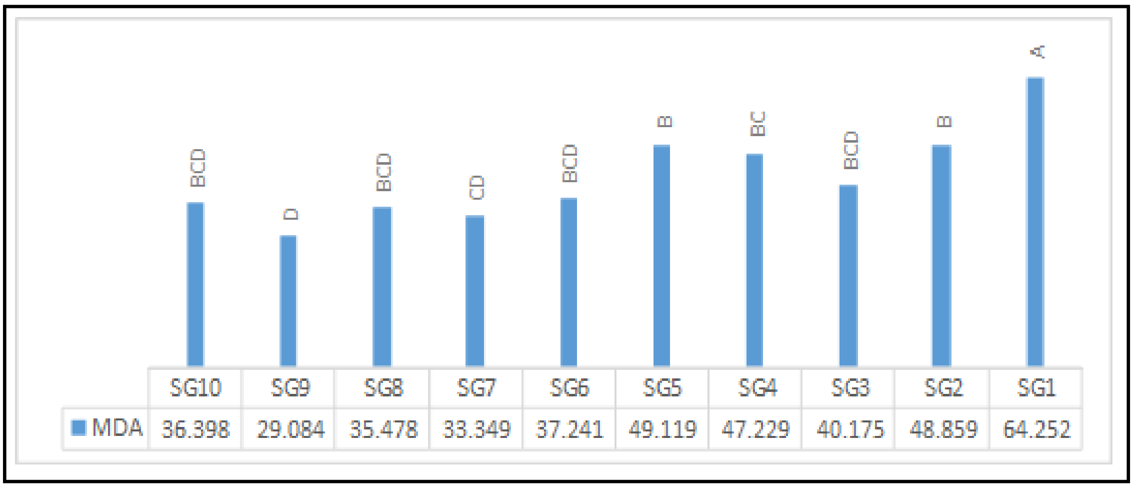

Figure 2: Effect of Sephadex filtration technique and adding of antioxidants on the malondialdehyde concentrations in seminal plasma of Holstein bulls.

SG1= poor semen quality (Tris); SG2= good semen quality (Tris); SG3= SG1+(glutamine-glycine) , SG4= SG1+ vit.E+Omega3; SG5 =Sephadex G15; SG6= SG5+ glutamine-glycine ; SG7=SG5+ vit.E-Omega3; SG8=Sephadex G25 ; SG9= SG8+ glutamine +glycine ; SG10= SG8+ vit.E+ omega3.

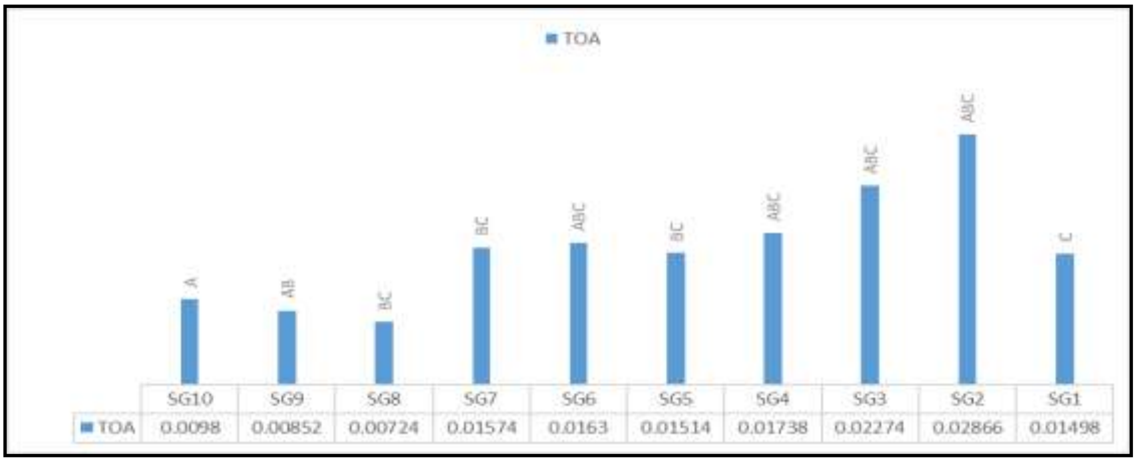

Figure 3: Effect of Sephadex filtration technique and addition of antioxidants on the total antioxidant concentrations in the seminal plasma of Holstein bulls.

SG1= poor semen quality (Tris); SG2= good semen quality (Tris); SG3= SG1+(glutamine-glycine), SG4= SG1+ vit.E+Omega3; SG5 =Sephadex G15; SG6= SG5+ glutamine-glycine ; SG7=SG5+ vit.E-Omega3; SG8=Sephadex G25 ; SG9= SG8+ glutamine +glycine ; SG10= SG8+ vit.E+ omega3.

Several types of Sephadex gel are produced, each designed for separating molecules based on size. These gels are classified into various grades (e.g., G-10, G-15, G-25, G-50, G-75, G-100, G-150, G-200), according to their molecular weight exclusion limits. The lowest grade, G-10, is suitable for molecules with molecular weights not exceeding 700 Daltons, while the highest grade, G-200, is designed for molecules ranging from 5,000 to 250,000 Daltons [7]. The current findings, which show improved semen quality in groups separated using Sephadex G-15 (SG5, SG6, and SG7) and Sephadex G-25 (SG8, SG9, and SG10) compared to the negative control group (SG1), are consistent with the results reported by Singh *et al*. [42]. In their study, a significant increase (P < 0.05) was observed in post-thaw individual motility (42.75 ± 1.02%) in buffalo bull semen filtered through Sephadex, compared to unfiltered semen (29.50 ± 0.85%). Additionally, the percentage of live sperm was significantly higher in the filtered group (52.90 ± 1.30%) than in the unfiltered group (41.60 ± 0.88%), and the proportion of morphologically normal sperm was also significantly greater (84.90 ± 0.48% vs. 74.35 ± 1.18%, respectively). Our results are in agreement with those of Januskauskas *et al.* [21], who reported that the use of Sephadex G-15 significantly enhanced sperm individual motility both after equilibration and post-cryopreservation. Additionally, a significant increase (P < 0.05) in the percentage of live sperm was observed following both equilibration and cryopreservation. The current findings are consistent with those reported by Husna *et al.* [8], who demonstrated an increase in progressive sperm motility post-cryopreservation in semen filtered with Sephadex G-15 (70 ± 2.23%) compared to unfiltered semen (57 ± 1.22%). Additionally, they observed a higher percentage of intact plasma membranes (75.6 ± 1.21% vs. 65.6 ± 1.17%), increased sperm vitality (67.6 ± 1.50%), and a significantly greater cleavage rate (44.5 ± 1.65%) in filtered semen compared to unfiltered semen (22.7 ± 1.67%). The current results are also in agreement with the findings of Alwaeli and Eidan [8], who reported significant improvements in semen quality following filtration with Sephadex G-15. Specifically, they observed a significant increase in individual sperm motility (36 ± 3.23%), live sperm percentage (74.4 ± 5.1%), normal sperm morphology (97.5 ± 0.17%), acrosome integrity (73.1 ± 4.55%), and plasma membrane integrity (73.8 ± 4.0%) in filtered buffalo bull semen, compared to the unfiltered samples, which exhibited lower values for each respective parameter (25.71 ± 2.39%, 61.07 ± 4.51%, 92.57 ± 0.50%, 56.32 ± 3.94%, and 55.1 ± 3.17%). These results highlight the efficiency of the Sephadex column in reducing the proportion of non-motile and dead sperm by selectively trapping them and preventing their passage through the column. This selective filtration reduces sources of free radical production and reactive oxygen species (ROS) [24], thereby enhancing the overall quality of the semen and improving its suitability for cryopreservation [19]. The present findings regarding vitamin E supplementation are consistent with those reported by Silva *et al.* [41], who demonstrated that the addition of Trolox, a water-soluble analog of vitamin E, to an egg yolk-based extender at concentrations of 60–120 μM significantly ($P \leq 0.05$) improved plasma membrane integrity, progressive sperm motility, and mitochondrial function in ram spermatozoa. Concomitantly, Zhaku *et al.* [4] reported a significant reduction ($P < 0.05$) in reactive oxygen species (ROS) concentrations following the addition of a vitamin E analogue, compared to the control group. In support of these findings, Prastiyaa *et al.* [35] demonstrated that supplementation with 1 mg/mL of α-tocopherol significantly improved ($P < 0.05$) sperm quality 24 hours post-thaw. Specifically, they observed increased individual motility (61.30 ± 3.88%) and live sperm percentage (77.85 ± 6.37%), along with a reduction in abnormal spermatozoa (3.41 ± 1.22%), compared to the control group values of 47.99 ± 2.85%, 71.22 ± 6.19%, and 5.41 ± 1.22%, respectively.

The current findings indicate that no significant differences were observed following supplementation with glycine and glutamine in groups SG3, SG6, and SG9 compared to their corresponding control groups, SG1, SG5, and SG8, respectively. These results are consistent with those reported by Nazif *et al.* [30], who investigated the effects of various concentrations of glycine on bull semen and concluded that supplementation with 20 mM glycine had no significant effect on total sperm motility, viability, or plasma membrane integrity. The lack of significant effects observed in the current study may be attributed to the possibility that the concentrations of glycine and glutamine used were insufficient to confer effective protection to bull spermatozoa under cryopreservation conditions.

Conclusion

In conclusion, sperm filtration proved to be an effective method for removing dead and abnormal spermatozoa, as well as cellular debris, particularly in bulls with low-quality ejaculates. This reduction in contaminants helps to minimize the sources of ROS during semen cryopreservation, thereby enhancing semen quality for both cryopreservation and artificial insemination. Additionally, supplementation of semen extenders with a combination of vitamin E and omega-3 fatty acids demonstrated protective effects against oxidative stress. In contrast, the supplementation of glycine and glutamine showed no significant impact on semen parameters under the conditions tested. Further research is warranted using different concentrations of these amino acids to determine their optimal levels for improving semen quality in Holstein bulls.

REFERENCES

1- Agarwal, A. and M.K.P. Selvam (2018). Advanced Sperm Processing/ Selection Techniques. In: Zini A, Agarwal A (eds.) A Clinician's Guide to Sperm DNA and Chromatin Damage. Springer, New York, pp. 529–543. DOI: 10.1017/9781108867245

2- Ahma, Z.; M. Anzar; M. Shahab; N. Ahmad and S. Andrabi (2003). Sephadex and Sephadex Ion-Exchange Filtration Improves the Quality And Freezability of Low-Grade Buffalo Semen Ejaculates. Theriogenology**.** DOI: 10.1016/s0093-691x(02)01159-7

3- Aitken, R.J. 1995. Free Radicals, Lipid Peroxidation And Sperm Function. Reprod. Fertil. Dev. 7: 659–668. DOI: 10.1071/rd9950659

4- Alwaeli, Saif N. and S. M. Eidan |(2023). Effect of Glass Wool and Sephadex Sperm Separation Techniques on Improving the Poor Quality Semen of Iraqi Buffalo Bulls. IOP Conf. Series: Earth and Environmental Science 1262,072003. DOI:10.1088/1755-1315/1262/7/072003

5- Alwaeli S. N. and S. M. Eidan (2024). Synergistic Effect Of Sperm Filtration Techniques And Antioxidants Added To Extender Of Iraqi Buffalo Semen Iraqi Journal Of Agricultural Sciences, 55(1):413–421. DOI: 10.36103/kc809146

6- Amidi, F.; A. Pazhohan; M.S. Nashtaei; M. Khodarahmian, S. Nekoonam, (2016). The role of antioxidants in sperm freezing: A review. Cell Tissue Bank., 17: 745–756 DOI: 10.1007/s10561-016-9566-5

7- Andrei, C.R.; F.P. Posastiuc; N.T. Constantin, and I.L. Mitrea (2024). New Insights into Semen Separation Techniques in Buffaloes. Frontiers in Veterinary Sciences. DOI: https://doi.org/10.3389/fvets.2023.1347482,

8- Asma-ul-Husna A.M.A.; A. Mehmood, Sultana, T. Shahzad, Q. Ansari, M.S. and Rakha, B.A. (2017). Sperm sexing in Nili-Ravi buffalo through modified swim up: validation using SYBR ® green real-time PCR. Anim. Reprod. Sci., 182:69–76 **.** DOI: 10.1016/j.anireprosci.2017.04.011

9- Azawi, O.I. and E.K. Hussein (2013). Effect of vitamins C or E supplementation to Tris diluent on the semen quality of Awassi rams preserved at 5 C. In Veterinary Research Forum; Faculty of Veterinary Medicine, Urmia University: Urmia, Iran,; p. 157. PMID: 25653790

10- Barbarosie, C.; M. Selvam and A. Agarwal (2021). Future Directives in Sperm Handling for ART. In A. Agarwal, A. Majzoub, & S. Esteves (Eds.), Manual of Sperm Retrieval and Preparation in Human Assisted Reproduction Cambridge University Press: 117-130 . DOI:10.1017/9781108867245.014

11- Beydola T.; R. K. Sharma and W. Lee (2013). Sperm Preparation and Selection Techniques. In: Rizk BRMB, Aziz N, Agarwal A, Sanbanegh E (eds.) Male Infertility Practice. Jaypee Brothers, New Delhi, pp: 244–251. DOI: 10.5005/jp/books/11840_29

12- Bucak, M.N.; P.B. Tuncer; S. Sariozkan and P. A. Ulutas (2009). Comparison of the Effects of Glutamine and an Amino Acid solution on post-thawed ram sperm parameters, lipid peroxidation and anti-oxidant activities. Small Rumin. Res., 81: 13–17. DOI: 10.1016/j.smallrumres.2008.10.003

13- Buettner, G.R. (1993). The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch Biochem Biophys. 300:535-543. DOI: 10.1006/abbi.1993.1074

14- Coyan, K.; M.N. Bucak; N. Baspinar; M. Taspinar and S. Aydos (2012). Ergothioneine attenuates the DNA damage of post-thawed Merino ram sperm. Small Rumin. Res., 106: 165–167. DOI:10.1016/j.smallrumres.2012.02.00

15- Eidan S. M., and S. A. Khudhir (2023). Association Between ATP1A``1 Gene Polymorphisms With Semen Characteristics In Holstein Bulls. Iraqi Journal Of Agricultural Sciences, 54(2):330-337. https://doi.org/10.36103/ijas.v54i2.1706`

16 Forouzanfar, M. Ershad, S.F. Hosseini, S.M. Hajian, M. Ostad-Hosseini, S. Abid, A. Tavalaee, M. Shahverdi, A. Dizaji, A.V. Esfahani, M.H.N. (2013). Can permeable super oxide dismutase mimetic agents improve the quality of frozen-thawed ram semen? Cryobiology, 66: 126–130. DOI: 10.1016/j.cryobiol.2012.12.006

17- Hancock, J. (1951). A staining technique for the study of temperature shock in semen. Nature, 167: 323-324. DOI: 10.1038/167323b0

18- Hassan, M. S. and S. M. Eidan (2021). Effect Of Swim-Up And Glass Wool Techniques, With Adding Antioxidants To Tris Extender On Improving Post-Cryopreserved Some Semen Attributes Of Low Semen Quality For Holstein Bulls. Iraqi Journal Of Agricultural Sciences, 52(3):552-563. https://doi.org/10.36103/ijas.v52i3.1342

19- Henkel RR, Schill WB. 2003. Sperm preparation for ART. Reprod Biol Endocrinol. DOI:10.1186/1477-7827-1-108

20- Hezavehei, M.; M. Sharafi; H.M. Kouchesfahani; R. Henkel; A. Agarwal V. Esmaeili and A. Shahverdi (2018). Sperm cryopreservation: A review on current molecular cryobiology and advanced approaches. Reprod. Biomed. Online, 37: 327–339. DOI: 10.1016/j.rbmo.2018.05.012

21- Januskauskas, A. Lukoseviciute, K. Nagy, S. Johannisson, A. and Rodriguez-Martinez, H. (2005). Assessment of the efficacy of sephadex g-15 filtration of bovine spermatozoa for cryopreservation. Theriogenology, 63(1):160-178. DOI: 10.1016/j.theriogenology.2004.04.002

22- Jeyendran, R. S.; H. H. Vander van; , M. B. Perez-Pelaez; G. Crabo, and L. J. D. Zaneveld (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil., 70: 219- 228. DOI: https://doi.org/10.1530/jrf.0.0700219

23- Kavak, A.; N. Lundeheim; M. Aidnik and S. Einarsson (2004|). Sperm morphology in Estonian and Tori breed stallions. Acta Vet Scand. 45:11-8. DOI: 10.1186/1751-0147-45-11

24- Kruk, J.; H. Y. Aboul-Enein,; A. Kładna and J. E. Bowser (2019). Oxidative Stress in Biological Systems and its Relation with Pathophysiological Functions: The Effect of Physical Activity on Cellular Redox Homeostasis. Free Radic. Res. 53(5):497–521. DOI: 10.1080/10715762.2019.1612059,

25- Love C.C. (2011). Relationship Between Sperm Motility, Morphology and the Fertility of Stallions. Theriogenology 76:547–557**.** DOI: 10.1016/j.theriogenology.2011.03.007

26- Menon AG, Barkema HW, Wilde R, Kastelic JP, Thundathil JC. 2011. Associations between sperm abnormalities, breed, age, and scrotal circumference in beef bulls. Can J Vet Res, 75:241-247 .PMID: 22468020

27- Minucci, S. and Venditti, M. 2022. New Insight on the in Vitro Effects of Melatonin in Preserving Human Sperm Quality. Int. J. Mol. Sci.,23, 5128. DOI: 10.3390/ijms23095128

28- Muratori, M. Tarozzi, N. Carpentiero, F. Danti, S. Perrone, F.M. Cambi, M. Casini, A. Azzari, C. Boni, L. Maggi, M. 2019. Sperm selection with density gradient centrifugation and swim up: Effect on DNA fragmentation in viable spermatozoa. Sci. Rep., 9, 7492. DOI: 10.1038/s41598-019-43981-2

29- 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. DOI:10.36103/6erkrp95

30- Nazif M.S. Rehman Z.U. Khan H. Khan F.A. Hussain T. Ahmad A. Farmanullah, Husnain A. Muhammad S. Murtaza G. and Gang L. 2022. Glycine Improved Cryopreserved Spermatozoa Quality in Achai Bull. Biomed Res Int. 4: 8282387. DOI:10.1155/2022/8282387

31- Ombelet W, Van Robays J. 2010. History of human artificial insemination. Facts, Views Vis OBGYN;1–5. Monograph. PMCID: PMC4498171

32- Ortiz, I. Y.; J. Catalán; Joan E. Rodríguez- G. Miró j. and Yeste M. 2022. Advances in sperm cryopreservation in farm animals: Cattle, horse, pig and sheep . Animal Reproduction Science *246, 106904*. DOI: 10.1016/j.anireprosci.2021.106904

33- Pezo, F. Zambrano, F. Uribe, P. Moya, C. de Andrade, A.F.C. Risopatron, J. Yeste, M. Burgos, R.A. and Sanchez, R. 2021. Oxidative and Nitrosative Stress in Frozen-Thawed Pig Spermatozoa. I: Protective effect of melatonin and butylhydroxytoluene on sperm function. Res. Vet. Sci., 136: 143–150. DOI: 10.1016/j.rvsc.2021.02.006

34- Pintus, E.; Ros-Santaella, J.L. 2021. Impact of Oxidative Stress on Male Reproduction in Domestic and Wild Animals. Antioxidants, 10:1154. DOI: 10.3390/antiox10071154

35- Pool, K.R. Rickard, J.P. and de Graaf, S.P. 2020. Melatonin improves the motility and DNA integrity of frozen-thawed ram spermatozoa likely via suppression of mitochondrial superoxide production. Domest. Anim. Endocrinol., 74, 8. DOI: 10.1016/j.domaniend.2020.106516

36- Prastiya,R. Rimayanti, A. Munir,M. M. and NugrohoA. P. 2021. The Protective Impacts of α-tocopherol Supplementation on the Semen Quality of Sapera Goat Preserved at 4°C. Tropical Animal Science Journal, 44(3): 261-266 DOI: 10.5398/tasj.2021.44.3.261

37- Rietjens, I. Boersma, M.G. de Haan, L. Spenkelink, B. Awad, H.M. Cnubben, N.H.P. van Zanden, J.J. van derWoude, H. Alink, G.M. and Koeman, J.H. 2002. The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. Environ. Toxicol. Pharmacol., 11: 321–333. DOI: 10.1016/s1382-6689(02)00003-0

38- Rodriguez-Martinez, H. Martinez, E.A. Calvete, J.J. Peña Vega, F.J. Roca, J. 2021. Seminal Plasma: Relevant for Fertility? Int. J. Mol. Sci., 22, 4368. DOI: 10.3390/ijms22094368

39- Sauer, R.; C. B. Coulam and R. S. Jeyendran (2012). Chromatin intact human sperm recovery is higher following glass wool column filtration as compared with density gradient centrifugation. *Andrologia*, *44*(Suppl 1): 248–251. DOI: https://doi.org/10.1111/j.1439-0272.2011.01171.x,

40- Sharafi, M.; S.M. Borghei-Rad; M. Hezavehei, A.Shahverdi, and J.D. Benson, (2022). Cryopreservation of Semen in Domestic Animals: A Review of Current Challenges, Applications, and Prospective Strategies. *Animals* , *12*, 3271. DOI:10.3390/ani12233271

41- Silva, S. V.; A. T. Soaresa; A. M. Batista; F. C. Almeidaa; J.F. Nunesd, Peixotoe, C. A. and M. M. P. Guerraa (2013). Vitamin E (Trolox) addition to Tris-egg yolk extender preserves ramspermatozoon structure and kinematics after cryopreservation Animal Reproduction Science 137: 37–44. DOI: 10.1016/j.anireprosci.2012.12.002

42- Singh, S.; G. R. Pangaonkar; K. C. Choudhary, and H. K. Verma (2002). Improvement of Buffalo Bull Semen Quality Through Sephadex Filtration and Subsequent Assessment Through Polyacrylamide Gel Column Migration. Indian Journal of Animal Reproduction. *Reprod., 23(2)*. https://acspublisher.com/journals/index.php/ijar/article/view/5943

43- Srivastava, N. and M. Pande (2017). Semen Analysis: An Overview. Protocols in Semen Biology (Comparing Assays): 1-6. DOI: 10.1007/978-981-10-5200-2

44- Su, G.H.; S.S. Wu; M.L. Wu, Wang; L.N. Yang, L. Du, M.X. Zhao, X.Y. X.H. Su; X.F. Liu and C.L. Bai (2021). Melatonin Improves the Quality of Frozen Bull Semen and Influences Gene Expression Related to Embryo Genome Activation. Theriogenology, 176: 54–62. DOI: 10.1016/j.theriogenology.2021.09.014

45- Tamburrino, L.; G. Traini; A. Marcellini; L. Vignozzi; E. Baldi and S. Marchiani (2023). Cryopreservation of Human Spermatozoa: Functional, Molecular and Clinical Aspects. International Journal of Moleculare Sciences, 24, 4656. DOI: 10.3390/ijms24054656,

46- Tsakmakidis, I.A. Lymberopoulos A.G. and Khalifa T.A. (2010). Relationship Between Sperm Quality Traits and Field-Fertility of Porcine Semen. Journal of Veterinary Sciences, 11:1514. DOI: 10.4142/jvs.2010.11.2.151

47- Usuga, A.; I. Tejera; J. Gomez; O. Restrepo; B. Rojano and G. Restrepo (2021). Cryoprotective Effects of Ergothioneine and Isoespintanol on Canine Semen. Animals, 11: 2757. DOI: 10.3390/ani11102757

48- Zhaku, V.; A. Agarwal; R. Henkel; R. Finelli; S. Beadini and S. Micic, (2021). Male Infertility, Oxidative Stress and Antioxidants. Vitamin E in Health and Disease - Interactions, Diseases and Health Aspects. IntechOpen. Male Infertility, Oxidative Stress and Antioxidants | IntechOpen

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تأثير ترشيح النطف بالسيفادكس واضافة مضادات اكسدة في صفات السائل المنوي بعد الاسالة لثيران الهولشتاين

حمزة يوسف علي[1] ساجدة مهدي عيدان[2]
Omaradel19812007@yahoo.com sajeda.mahdi@coagri.uobaghdad.edu.iq
عمر عادل محمد[1]
omaradel19812007@yahoo.com

الملخص

هدفت الدراسة الحالية للكشف عن تأثير ترشيح السائل المنوي بدرجتين من السيفادكس لتحسين نوعية السائل المنوي رديء النوعية للثيران بعد الحفظ بالتجميد مع إضافة مضادات الأكسدة. تم جمع عينات السائل المنوي من ثمانية ثيران من نوع هولشتاين على مدى فترة ثمانية أسابيع، وتم تقسيمها إلى أربع مجموعات تجريبية. شملت المجموعة الأولى (SG5، SG6، SG7) ترشيح النطف رديئة النوعية باستعمال مادة السيفادكس G-15. أما المجموعة الثانية (SG8، SG9، SG10)، فقد تم فيها ترشيح النطف الرديئة التوعية باستخدام السيفادكس G-25، في حين تألفت المجموعة الثالثة (SG1) من سائل منوي رديء النوعية غير مُرشَّح، وقد استُخدمت كمجموعة سيطرة سالبة. تم تخصيص مجموعة من عينات السائل المنوي ذات النوعية الجيدة (SG2) كمجموعة سيطرة موجبة. تم دعم المجاميع SG3، SG6، وSG9 بمزيج من الحامضين الأمينين الكلايسين (20 ملي مول) والكلوتامين (1 ملي مول)، في حين دعمت المجاميع SG4، SG7، وSG10 بمقدار 0.2 ملي مول من فيتامين E و0.8 ملغم/مل من الأحماض الدهنية أوميكا-3. أما المجموعتان SG5 وSG8، اللتان خضعتا لعملية الترشيح دون أي دعم إضافي، فقد استخدمتا كمجموعتي سيطرة فرعية. أظهرت النتائج تحسنًا معنويًا ($P \le 0.01$) في حركة النطف وحيويتها وشكلها الطبيعي وسلامة الأكروسوم، والغشاء البلازمي في المجاميع التي خضعت للترشيح باستخدام مادة السيفادكس (SG5، SG6، SG7، SG8، SG9 وSG10)، بعد مرحلتي التبريد والحفظ بالتجميد. اضافةً الى ذلك، أظهرت المجاميع SG4، SG7 وSG10 تحسنًا معنويًا ($P \le 0.01$) في حركة النطف وحيويتها، وسلامة غشائها البلازمي مقارنةً بمجموعتي السيطرة SG8 وSG1. ويمكن الاستنتاج أن ترشيح النطف يُعدّ وسيلة فعّالة في تقليل نسبة النطف الميتة وغير الطبيعية، فضلاً عن الحطام الخلوي، في القذفات الرديئة النوعية لثيران الهولشتاين.

الكلمات الدالة: الثيران، مضادات الاكسدة، السيفادكس، التجميد، ترشيح النطف.

[1] جامعة الانبار، كلية الزراعة، الانبار، العراق.
[2] جامعة بغداد، كلية علوم الهندسة الزراعية، بغداد، العراق.

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