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The effect of Sil-select and swim-down techniques with antioxidant added to diluent on buffalo bull's semen traits

The effect of Sil-select and swim-down techniques with antioxidant added to diluent on buffalo bull's semen traits

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Abstract: A study investigated the effect of sil-select and swim-down sperm selection protocols on enhancing the bad semen attributes of buffalo bulls with or without antioxidants. Semen was evaluated weekly (14 weeks) and divided into 12 groups. Good (GSQ) and bad (BSQ) semen were diluted using a Tris diluent. The GSQ was divided into three groups [CS1: Tris diluent; CS5: Tris+ vitamins E (2 mmol) and C (5 mmol); CS6: Tris +glutamine (20mmol) and arginine (1mmol)]. The BSQ was divided into three groups and three sub-groups (CS2: Tris diluent; CS7: Tris +vitamins E and C; CS8: Tris+ glutamine and arginine). In the 3rd and 4th main groups, the Sil-Select (CS3) and swim-down (CS4) techniques were used with or without adding antioxidants and subdivided into three sub-groups, referred to as CS9-CS10 for the sil-select method and CS11-CS12 for the swim-down. Improving normal morphology (NM), acrosome, plasma membrane integrity and lesser sperm abnormalities (SAB) were noticed in fresh semen of the CS3 than the CS2 groups. The CS3, CS4, and CS9-CS12 groups recorded higher NM and lower SAB than other groups post-cryopreservation. The two techniques removed SAB and harvested good sperm, which may improve the pregnancy rate and agricultural sustainability systems.

1. Introduction

The livestock sector is a power source for global development and economic growth. Agriculture must use non-utilized but widely available natural resources like food-producing plants and animals for the new sustainable development goals. Semen quality plays a pronounced role in the successfulness artificial insemination (AI) and the enhancement of genetic potential in livestock species [1]. However, there are inherent problems with semen quality in buffalo bulls, including poor freezability and overall low quality [2,3,4], which leads to genetic and economic losses because of culling bulls of high genetic value [5]. The main factors influencing semen quality are the season (Photoperiod, humidity, temperature) and nutrition (forage availability), which affect reproductive performance. [6]. In addition to seasonal effects, vaccination protocols for exotic and crossbred animals can also harm semen quality [6,7,8]. Hybrid animals are more prone to contagious diseases, often prevented through vaccination.

Sperm selection techniques have been utilized to obtain the maximum genetic outcome from superior bulls and maximize the production of high-quality semen without discarding too many poor-quality ejaculates. The use of selection techniques like dilution and washing [9], separation filters [glass wool and Sephadex; 4, 10–11], density gradient techniques [sil-select; 12], bovipure [13], and self-migration (swim-up or swim-down) techniques [10–12] had been found to significant ameliorate sperm quality at fresh, post-thaw, and fertility rates [11, 14–15].

Another approach for ameliorating semen quality is the addition of antioxidants [3,10,17-20]. Antioxidant fortification with substances like vitamins E and C has positively impacted motility, viability, acrosome integrity of sperm, and other semen characteristics [14,16,21-23]. Glutamine and arginine play antioxidant roles [2], protecting sperm from freezing damage in bulls [24-25]. Improving semen quality in buffalo bulls is crucial for achieving breeding goals and maximizing genetic potential through AI programs [2]. By implementing selection techniques and fortifying with antioxidants, sound improvements can be made in the semen quality of buffalo bulls [2]. These will ultimately lead to higher conception rates and improved fertility in buffalo females, allowing AI to be widely used in buffalo mating programs.

There have been no studies on semen filtration techniques for low-quality semen from buffalo bulls in Iraq. This study investigated the effects of using Sil-Select and swim-down techniques to filter low-quality semen from Iraqi buffalo bulls. The addition of antioxidants (vitamin C with E or glutamine with arginine) may protect or activate the filtered or unfiltered semen through the cryopreservation process for buffalo bulls. These could make it possible to preserve the semen by freezing and using it for traditional purposes.

2. Materials and Methods

An experiment was undertaken at the AI Department. Ten good-health Iraqi buffalo bulls (500–750 kg and 6–8 years) were used for semen collection. The AI Department, Directorate of Animal Resource, Ministry of Agriculture, Iraq does not require ethical approval for reporting individual cases or cases series because they subjected animals under continuous veterinary and management care. All procedure in this study were conducted in accordance with Ministry of Agriculture approved protocol. Bulls with bad (BSQ; progressive motility less than 40%) and good (GSQ; progressive motility more than 50%) semen were collected weekly at one ejaculate / bull for 14 weeks. Both types of semen were split into 12 groups using a Tris diluent. The GSQ was split into three groups: [CS1: Tris diluent; CS5: Tris+ vitamins E (2 mM) and C (5 mM); CS 6: Tris +glutamine (20 mM) and arginine (1 mM)]. Bad semen was split into three groups and sub split into three subgroups (CS2; Tris diluent; CS7; Tris +vitamins E (2 mM) and C (5 mM); and CS8; Tris + glutamine (20 mM) and arginine (1 mM). In the 3rd and 4th main groups, the sil-select (CS3) and swim-down (CS4) techniques were used with or without adding vitamins (E+C) and amino acids (glutamine and arginine) and subdivided into three subgroups or processes related to CS9-CS10 for sil-select and CS11-CS12 for swim-down techniques. All buffalo bulls were allocated to a standardized diet, as a concentrate ration (protein 18% and 2146 kcal) was provided daily at a rate of 4-6 kg/bull. Roughage consisted of alfalfa hay (7-9 kg/bull/day) and green forage (50–60 kg/bull/day). Salt blocks and freshwater were available *ad libitum* to the bulls.

The semen characteristics were evaluated [26] fresh, post-cooling, and cryopreservation (motility, live, normal morphology, integrity of membrane plasma and acrosome, total abnormalities, total antioxidants concentrations, malondialdehyde concentrations, damage of DNA sperm, and freezability).

Data were computed using the SAS software based on CRD to explore the influence of sperm selection protocols on the study's parameters. Significant differences were compared using Duncan's multiple range test.

3. Results and Discussion

3.1. Fresh

Groups CS1 and CS2 recorded the highest ($P \leq 0.01$) concentration of sperm compared with the CS3 and CS4 groups (Table 1). The CS1 group revealed more elevated motility of sperm cells in comparison with the CS2 and CS4 groups. Sil-select (CS3) and swim-down (CS4) showed ($P \leq 0.01$) the higher normal sperm (%) compared with groups CS1 and CS2, while group CS2 recorded the lowest normal sperm (%) compared with the CS1 and CS3-CS4 groups. The acrosome and plasma membrane integrity percentages were greater ($P \leq 0.01$) in the CS1 than in the CS2-CS3 groups. The Sil-select group (CS3) recorded greater ($P \leq 0.01$) integrity of the acrosome and plasma membrane than the negative control group (CS2). Group CS1 gave the highest ($P \leq 0.01$) percentage of live as compared with the CS2-CS4

group. The live sperm percentage was increased in the CS4 group in comparison with the CS2 group. The total abnormal sperm declined ($P \leq 0.01$) in Sil-select (CS3) and Swim-down (CS4) compared to groups CS1 and CS2 (Table 1).

Table 1. Effect of Sil-select and swim-down sperm selection techniques on some fresh semen traits of Buffalo bulls.

Traits	Groups				Sig.
	CS1	CS2	CS3	CS4	
Concentration($\chi 10^6$ /ml)	$889.1^{a}\pm81.4$	$943.7^{a}\pm79.6$	$447.6^{b}\pm17.2$	$594.9^{b}\pm55.4$	P≤0.01
Motility (%)	$51.4^{a}\pm0.6$	$40^{b}\pm1.05$	$40^{b}\pm2.3$	$41.3^{b}\pm3.4$	P≤0.01
Normal morphology (%)	$95.1^{b}\pm0.2$	$91.8^{c}\pm0.3$	$97.9^{a}\pm 0.2$	97.5 a ±0.4	P≤0.01
Acrosome integrity (%)	$82.2^{a}\pm1.9$	$64.5^{c}\pm1.6$	$72.7^{b}\pm1.9$	$69.4^{bc}\pm2.4$	P≤0.01
Plasma membrane integrity (%)	$82.4^{a}\pm2.2$	$64.2^{c}\pm1.8$	$74.3^{b}\pm2.8$	$68.1^{bc}\pm3.1$	P≤0.01
Live sperm (%)	$85.6^{a}\pm1.4$	$68.6^{c}\pm1.0$	$73.4^{bc}\pm2.9$	$74.3^{b}\pm2.2$	P≤0.01
Total abnormalities (%)	$4.9^{b}\pm0.2$	$8.2^{a}\pm0.3$	$2.3^{c}\pm0.2$	$2.5^{c}\pm0.4$	P≤0.01

CS1 = Semen good quality; CS2 = Semen bad quality; CS3= Sil-select selection technique; CS4= Swim-down selection technique.

Table 2. Effect of Sil-select and swim-down techniques for sperm selection with or without adding antioxidants on some of semen traits in Iraqi buffalo bull's post- cooling (mean ± standard error)

Groups	Traits (%)					
	Motility	Normal Morphology	Live sperm	Acrosome Integrity	Plasma membrane integrity	Total Abnormalities
CS1	$40^{a}\pm2.3^{a}$	$95.2^{c}\pm0.3$	$72.3^{ab}\pm2.9$	$66.3^{ab}\pm2.7$	$65.7^{ab}\pm2.7$	$4.8^{b}\pm0.3$
CS2	$34.3^{abc}\pm1.4$	$92.2^{d}\pm0.5$	$65.5^{abcd}\pm1.5$	$65.2^{ab}\pm1.8$	$63.4^{ab}\pm1.9$	$7.8^{a}\pm0.5$
CS3	$35^{abc}\pm2.3$	$97.4^{ab}\pm0.2$	$69^{abc}\pm4.6$	$62.6^{abc}\pm3.4$	$64.3^{ab}\pm3.1$	$2.6^{c}\pm0.4$
CS4	$30^{c}\pm1.3$	$97.7^{ab}\pm0.2$	$57.5^{d}\pm2.6$	$51.6^{d}\pm2.4$	$49.6^{d}\pm3.4$	$2.3^{cd}\pm0.2$
CS5	$39.2^{a}\pm3.1$	$95.4^{c}\pm 0.3$	$70.3^{abc}\pm4.5$	$64.3^{ab}\pm4.5$	$63.6^{ab}\pm4.6$	$4.6^{b}\pm0.3$
CS6	$40^{a}\pm2.1$	$95.2^{c}\pm 0.4$	$74.3^{a}\pm3.2$	$70.6^{a}\pm3.2$	$70^{a}\pm3.2$	$4.8^{b}\pm0.4$
CS7	$36.4^{a}\pm1$	$93.1^{d}\pm 0.3$	$69.7^{abc}\pm1.3$	$66.1^{ab}\pm1.4$	$66.5^{ab}\pm1.7$	$6.9^{a}\pm0.3$
CS8	$35^{abc}\pm1$	$92.9^{d}\pm0.4$	$66.1^{abcd}\pm1.8$	$64^{ab}\pm2.2$	63.57 ± 2.52^{ab}	$7.1^{a}\pm0.4$
CS9	$32.5^{bc}\pm2.1$	$98.2^{ab}\pm0.4$	$63.5^{bcd}\pm3.4$	$61.4^{abc}\pm2.3$	$61.8^{abc}\pm2.8$	$1.8^{cd}\pm0.4$
CS10	$30^{c}\pm1.3$	$97.9^{ab}\pm 0.3$	$63.6^{bcd}\pm 3.4$	$62.7^{abc}\pm2.1$	$62.1^{abc}\pm3.2$	$2.1^{cd}\pm0.3$
CS11	$32.5^{bc}\pm1$	$98.5^{ab}\pm0.1$	$61.3^{cd}\pm 2.3$	$54.3^{cd}\pm3.1$	$52.7^{cd}\pm3.8$	$1.5^{cd}\pm0.1$
CS12	$35^{abc}\pm1.3$	$98.8^{a}\pm0.1$	$70.2^{abc}\pm3.1$	$59.8^{bcd}\pm2.6$	$57.1^{bcd}\pm3.6$	$1.3^{d}\pm0.1$
Sig.	P≤0.01	P≤0.01	P≤0.05	P≤0.01	P≤0.01	P≤0.01

CS1 = Semen good quality + Tris diluent; CS2 = Semen bad quality + Tris diluent; CS3 = Sil-select separation technique + Tris diluent; CS4= Swim-down separation technique + Tris diluent; CS5= CS1 with vitamin E (2 mmol) + vitamin C (5 mmol); CS6= CS1 with glutamine (20 mmol) + arginine (1 mmol); CS7= CS2+ Vitamin E + C; CS8= CS2 + with glutamine + arginine; CS9= CS3 with vitamin E + vitamin C; CS10= CS3 + glutamine + arginine; CS11 = CS4 + vitamin E + C; CS12 = CS4 + glutamine + arginine.
CS1, CS6, CS10 and CS12 (Figure 1 D).

3.2. Post-cooling

The CS1 and CS5 - CS7 groups showed higher ($P \leq 0.01$) progressive sperm motility than the CS4, CS9-CS11 groups post-cooling (Table 2). A swim-down (CS4, CS11-CS12) and Sil-select (CS3, CS9-CS10) with or without antioxidants groups gave the highest ($P \leq 0.01$) percentage of normal sperm compared to groups CS1–CS2 and CS5-CS8(Table 2). The results exhibited a significant difference in the

percentages of live sperms, sperm's acrosome, and plasma membrane integrity of sperm post-cooling among groups of the study.

The CS6 group gave the greatest values of live sperm, integrity of sperm's acrosome, and plasma membrane, while the CS4 revealed the lowest rate (Table 2). The swim-down (CS4, CS11-CS12) and sil-select (CS3, CS9-CS10) with or without antioxidants groups recorded the lowest (P≤ 0.01) total abnormalities percentages (Table 2) than other groups (CS1-CS2 and CS5-CS8).

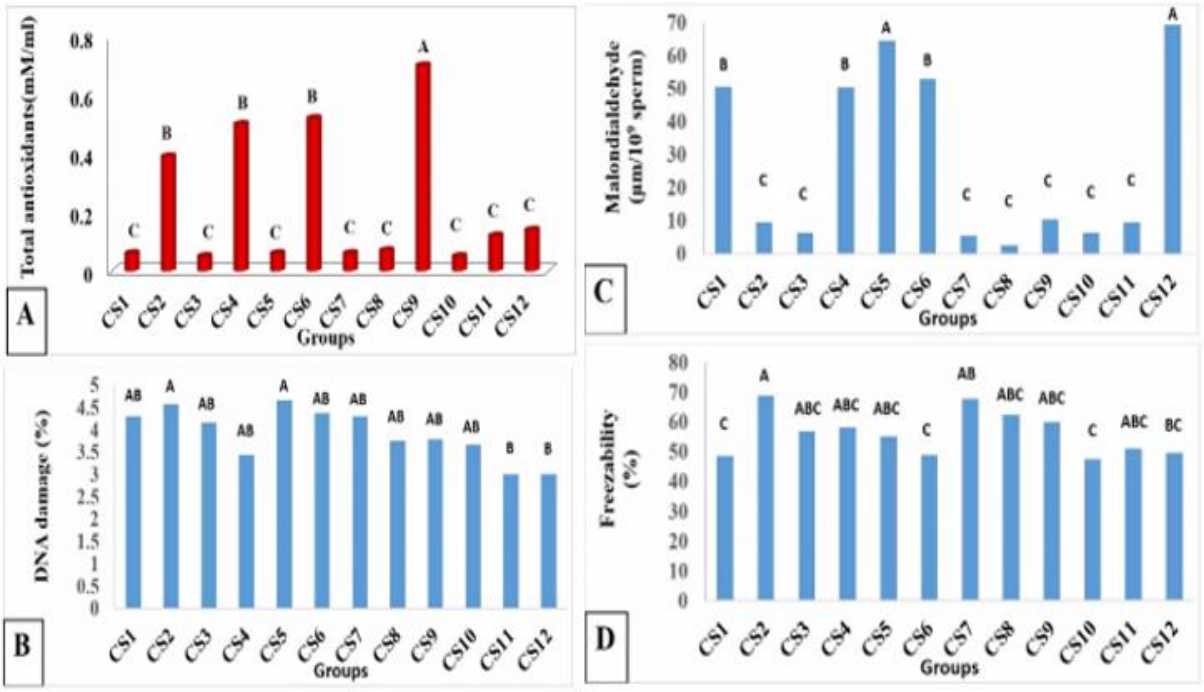

Figure 1. Effect of Sil-select and swim-down selection techniques with or without adding antioxidants on some of semen traits in Iraqi buffalo bull's post-cryopreservation.

Different superscripts among column indicated significant differences (P ≤ 0.01) among groups. CS1 = Semen good quality + Tris diluent; CS2 = Semen bad quality + Tris diluent; CS3 = Sil-select separation technique + Tris diluent; CS4= Swim-down separation technique + Tris diluent; CS5= CS1 with vitamin E (2 mmol) + C (5 mmol); CS6= CS1 with glutamine (20 mmol) + arginine (1 mmol); CS7= CS2+ Vitamin E + C; CS8= CS2 + with glutamine + arginine; CS9= CS3 with vitamin E + C; CS10= CS3 + glutamine + arginine; CS11 = CS4 + vitamin E + C; CS12 = CS4 + glutamine + arginine.

3.3. Post- cryopreservation

The groups CS2, CS5, and CS7 demonstrated greater progressive sperm's cell motility post-cryopreservation compared to the CS10-CS12 groups (Table 3). The swim-down (CS3, CS9-CS10) and sil-select (CS4, CS11-CS12) with or without antioxidants recorded the highest (P ≤ 0.01) sperm normal morphology (%) post- cryopreservation compared to the rest of the groups(CS1-CS2,CS5-CS6, CS7-CS8). Groups CS2, CS5, and CS7 showed a difference in live sperm percentage compared to the CS10-CS12 (Table 3). The results revealed pronounced variation among the CS5 group with CS4 and CS11 groups in acrosome integrity (%). The groups CS5 and CS7 recorded the highest plasma membrane integrity percentages compared to the CS4, CS10, and CS11 groups. The CS3-CS4, CS9-CS10, and CS11-CS12 groups recorded the lowest (P ≤ 0.01) sperm normal morphology (%) post- cryopreservation compared to the other groups (CS1-CS2, CS5-CS6, CS7-CS8) post- cryopreservation (Table 3).

The results showed that group sill-select with vitamins (CS9) had the highest (P ≤ 0.01) total antioxidant activity compared to all other groups. In contrast, groups CS2, CS4, and CS6 recorded an increase (P ≤ 0.01) in total antioxidant activity compared to groups CS1, CS3, CS5, CS7, CS8, and CS10-CS12, which recorded the lowest (P ≤ 0.01) total antioxidant activity (Figure 1 A). Groups Swim-

down with Vitamins (CS11) and Swim-down with amino acids (CS12) recorded the lowest ($P \leq 0.01$) DNA damage compared to groups CS2 and CS5 (Figure 1 B). A obvious decrease in malondialdehyde concentration in groups CS2, CS3, and CS7-CS11 compared to the rest of the groups, also a reduction ($P \leq 0.01$) in groups CS1, CS4, and CS6 in comparison with CS5 and CS12 groups, which recorded the highest ($P \leq 0.01$) malondialdehyde concentration (Figure 1 C). Groups CS2 and CS7 gave the more significant ($P \leq 0.01$) freezability, while the lower ($P \leq 0.01$) freezability was found in groups.

Table 3. Effect of Sil-select and swim-down techniques for sperm selection with or without adding antioxidants on some of semen traits in Iraqi buffalo bull's post- cryopreservation

Groups	Traits (%)					
	Motility	Normal Morphology	Live sperm	Acrosome Integrity	Plasma membrane integrity	Total Abnormalities
CS1	$25^{ab}\pm2.6$	$79.5^{d}\pm0.4$	$49.7^{ab}\pm3.4$	$49.8^{ab}\pm2.5$	$49.0^{ab}\pm2.7$	$20.5^{b}\pm0.4$
CS2	$27.9^{a}\pm1.6$	$76.9^{e}\pm0.3$	$54.7^{a}\pm2.7$	$50.1^{ab}\pm2.4$	$49.1^{ab}\pm2.9$	$23.1^{a}\pm0.3$
CS3	$22.5^{ab}\pm1.6$	$82.3^{bc}\pm0.4$	$46.6^{ab}\pm2.7$	$42.6^{ab}\pm2.8$	$40.9^{ab}\pm3.7$	$17.8^{cd}\pm0.4$
CS4	$22.5^{ab}\pm3.1$	$81.8^{c}\pm0.4$	$43.2^{b}\pm0.8$	$40.3^{b}\pm1.8$	$39.1^{b}\pm2.2$	$18.2^{c}\pm0.4$
CS5	$28.3^{a}\pm2.4$	$80.1^{d}\pm0.3$	$55.4^{a}\pm2.5$	$53.3^{a}\pm2.4$	$52.3^{a}\pm2.6$	$19.9^{b}\pm0.3$
CS6	$25^{ab}\pm3.0$	$79.1^{d}\pm0.3$	$45.6^{ab}\pm2.3$	$44.4^{ab}\pm2.4$	$44.3^{ab}\pm2.3$	$20.9^{b}\pm0.3$
CS7	$27.1^{a}\pm1.6$	$77.4^{e}\pm0.4$	$55.8^{a}\pm2.6$	$50.3^{ab}\pm2.7$	$51.2^{a}\pm2.6$	$22.6^{a}\pm0.4$
CS8	$25^{ab}\pm1.8$	$77.2^{e}\pm0.4$	$50^{ab}\pm2.4$	$48.2^{ab}\pm2.8$	$47^{ab}\pm3.1$	$22.8^{a}\pm0.4$
CS9	$25^{ab}\pm3.5$	$82.9^{abc}\pm0.3$	$49.3^{ab}\pm6.1$	$46.9^{ab}\pm5.7$	$44.9^{ab}\pm5.6$	$17.1^{cde}\pm0.3$
CS10	$18.8^{b}\pm1.6$	$83.4^{a}\pm 0.3$	$43.4^{b}\pm4.2$	$43.2^{ab}\pm4.0$	$39.8^{b}\pm3.4$	$16.6^{e}\pm0.3$
CS11	$18.8^{b}\pm2.5$	$83.3^{ab}\pm0.2$	$39.8^{b}\pm4.6$	$40.4^{b}\pm4.9$	$39.4^{b}\pm4.9$	$16.8^{de}\pm0.2$
CS12	$18.8^{b}\pm2.1$	$82.8^{abc}\pm0.4$	$41.8^{b}\pm4.2$	$43.3^{ab}\pm5.1$	$41.6^{ab}\pm5.2$	$17.2^{cde}\pm0.4$
Sign.	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.01$

CS1 = Semen good quality + Tris diluent; CS2 = Semen bad quality + Tris diluent; CS3 = Sil-select separation technique + Tris diluent; CS4= Swim-down separation technique + Tris diluent; CS5= CS1 with vitamin E (2 mmol) + C (5 mmol); CS6= CS1 with glutamine (20 mmol) + arginine (1 mmol); CS7= CS2+ Vitamin E + C; CS8= CS2 + with glutamine + arginine; CS9= CS3 with vitamin E + C; CS10= CS3 + glutamine + arginine; CS11 = CS4 + vitamin E + C; CS12 = CS4 + glutamine + arginine.

The growing population in the world and the climate changes that the world is witnessing, such as drought, lack of rain, and high temperatures, call on all researchers in the field of agricultural sustainability to work hard to exploit all natural plant and animal resources to produce food for humans. We noted in Table 1 the decrease in sperm concentration for the Sil-select (CS3) and swim-down (CS4) groups and the superiority of these two groups over the bad semen quality group (CS2) in normal morphology (%), sperm's acrosome and plasma membrane integrity percentages, sperm live (%) and decreased percentages of abnormal spermatozoa, as these two techniques prevent the passage of immotile, dead and abnormal sperm and thus lead to a decrease in semen concentration and improve sperm quality [27, 28]. The superiority of group CS1 over some other groups during the cooling and freezing period may be due to the semen quality used. Also, we noted the dominance of group CS3 in the sperm progressive motility, which may be returned to the role of the Sil-select technique in selecting high-quality sperm and getting rid of poor sperm, as sperm criteria such as motility, morphology, and DNA damage are important factors that affect pregnancy [29, 30]. Some researchers found that separating sperm using the Sil-select technique led to a notable increase in total motility of spermatozoa, reduced rates of abnormal spermatozoa, and immature chromatin significantly [31].

The sperm cryopreservation and thawing protocols leads to profound harmful changes in sperm functions [32]. Semen has some endogenous antioxidant reserve that scavenge the free radicles and arrest damage to the sperm [33]. The mis proportion between the ROS and antioxidant reserve in sperm can be regarded as a crucial reason behind the sperm damage by cryopreservation [34, 35]. Because

these antioxidants are insufficient [2], some are added to the semen to compensate for this deficiency and preserve the sperm from damage [14, 36]. Antioxidants act like a defense against oxidative stress [37]. Our results mention in Tables 1 and 2 the imitate of antioxidants in preserving spermatozoa damage during cooling and freezing as we find that groups CS5-CS8 and CS12 have maintained some sperm criteria.

Figure 1-A shows a decrease in antioxidant activity in some groups, possibly due to their use to protect DNA from impairment induced by free radicles [10,14,42]. Many investigations have been performed to determine the function of antioxidants that protect sperms from internal and external ROS and the harmful influences of the freezing process, and antioxidant types (catalase, vitamins C and E, lipoic acid, and glutathione) revealed that they protect sperm DNA from the effects of external ROS. [38, 39]. Despite the differences in DNA damage rates among groups in a current study, they have not yet exceeded the permissible limits (7-20%), a good indicator of Bull fertility [40]. We noted (Figure 1-B) that the swim-down technique with the addition of antioxidants has reduced DNA damage, as this technique separates immotile, dead, and abnormal sperm in addition to the complementary function of antioxidants to protect sperm from damage. A significant decrease in malondialdehyde concentration (Figure 1-C) may be due to sufficient amounts of total antioxidants. It can be concluded that the Sil-select plus density gradient separation technique provides a good tool for improving the quality of bad

Semen with or without adding antioxidants to the semen diluent. We find from Figure 1-D that most of the separation techniques groups with or without adding antioxidants (CS3, CS4, CS9, CS11, and CS12) in addition to the control groups with or without adding antioxidants (CS1-CS2, CS5, CS7 and CS8) have outperformed in freezability over the positive control group (CS1) as well as group CS6 and CS10, and this may be due to the complementary role of antioxidants in protecting sperm and improving their quality [41]. The lack of improvement in other characteristics of the buffalo semen selected by these techniques may be due to it taking a longer time due to the use of semen without diluting it before selection, which causes the spermatozoa to suffer from acidity in the selecting tubes as a result of the metabolic processes that it carries out before cooling and cryopreservation, which affects the sperm membrane integrity and motility. So, we need to reduce the selection time of the sperm, or the semen needs other antioxidants or to be treated with substances that modify the pH or increase the sperm's activation in the future.

4. Conclusion

The swim-up and sil-select sperm selection techniques removed sperm abnormalities and harvested an excellent sperm normal morphology, which may improve the pregnancy rate and agricultural sustainability systems by using non-utilized but widely available natural resources for increased food-producing by buffalo.

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